

TissueQuest 3.0

User Manual

Table of Contents

TissueQuest 3.0.....	Fehler! Textmarke nicht definiert.
1. Introduction	6
1.1. Purpose	6
1.2. Goals	6
1.3. Definitions, Acronyms and Abbreviations.....	6
1.4. Conventions.....	8
2. Installing TissueQuest.....	9
2.1. Application Dependencies.....	9
2.2. Windows 3.1 Installer for Windows XP SP2.....	9
2.3. Microsoft Visual C++ 2005 Redistributables for Windows XP	9
2.4. Intel Performance Primitives 5.3	9
2.5. MARX Dongle Drivers	9
2.6. System Requirements	10
2.6.1 Hardware Requirements	10
2.6.2 Software Requirements	10
3. Login to TissueQuest	11
4. Create new projects	12
4.1. Step 1	13
4.2. Step 2	14
4.3. Step 3	15
4.4. Step 4	16
4.4.1 Templates for the FOV' size (scaling).....	28
4.5. Step 5	29
5. Open Existing Project.....	30
5.1. Save Project	30
5.2. Print Project.....	31
6. Project Menu	32
6.1. Clean	32
6.2. Clean Temporary Files	32
6.3. Group of Channels	33
6.4. Virtual Channels	35
6.5. Individual Dots Settings	36
6.6. Properties	38
7. Main Window.....	39
7.1. Components of the Main Window	39
7.2. Menu.....	40
7.3. Toolbar	42
7.4. Parameters of algorithm	43
7.5. Channel list.....	44
7.6. Scattergram list.....	45
8. Detail Window	46
8.1. Detail Window: FOV Viewer Toolbar	49
8.2. Delete events.....	50
8.3. Split events	51
8.4. Merge events.....	51
8.5. Create events	52
8.6. Contextual menu	53
9. Samples	56
9.1. Select Sample to be Displayed	56
9.2. Sample linking	56
10. Scattergrams	60
10.1. Manage scattergrams.....	60
10.1.1 Add scattergram.....	60
10.1.2 Delete Scattergram	61
10.1.3 Show/Hide scattergram.....	62
10.2. Display settings	64

10.3.	Scattergram Toolbar.....	66
10.4.	Cutoffs	67
10.4.1	Add cutoff	67
10.4.2	Modify cutoff	68
10.4.3	Delete cutoff	69
10.4.4	Copy cutoff	70
10.4.5	Paste cutoff	71
10.5.	Set gates	72
10.5.1	Add rectangular gate.....	72
10.5.2	Polygon gating method	74
10.5.3	Modify gate.....	75
10.5.4	Delete gate.....	77
10.5.5	Gate Management	79
10.6.	Scattergram parameters.....	81
10.7.	View backward data	82
11.	Forward and backward connection.....	83
11.1.	Forward connection	83
11.2.	Backward connection	84
12.	Project properties.....	87
12.1.	Samples.....	88
12.2.	Representative/Non-representative FOVs	88
12.3.	Markers.....	90
12.4.	FOV Size	91
13.	Evaluation	93
13.1.	Experiment evaluation	93
13.2.	Scattergram evaluation	93
14.	Special features	94
14.1.	Print project	94
14.2.	Export	95
15.	Segmentation Methods.....	98
15.1.	Changing Segmentation Method.....	99
15.2.	Various Shapes 1.0	99
15.2.1	Master Channel Parameters	99
15.2.2	Non-Master Channel Parameters	100
15.3.	DOT Finder.....	101
15.3.1	DOT Parameters	101
16.	Image Analysis	103
17.	Analysis Results	105
17.1.	Image Results.....	105
17.2.	Raw Data.....	106
17.2.1	Raw Data for Various Shapes 1.0.....	106
17.2.2	Raw Data for DOT Finder	108
18.	Admin menu.....	109
18.1.	Users	109
18.2.	Institution Data.....	112
18.3.	Change Password	112
19.	Help menu.....	114
20.	References.....	Fehler! Textmarke nicht definiert.
21.	Document History	Fehler! Textmarke nicht definiert.
22.	Document Quality Assurance	Fehler! Textmarke nicht definiert.

Disclaimers

1. **TissueFAXSplus** is a microscope-based cell analysis system for cells in cryocuts, paraffin sections and TMAs. It consists of the software modules "**TissueFAXS**", "**TissueQuest**", and "**HistoQuest**" and is used for acquisition of images in the fluorescence and brightfield mode, for counting the number of positive and negative cells and for quantification of staining intensities. **TissueFAXSplus** is used for the standardization of tissue analysis in combination with immunohistochemical and immunofluorescence staining.

The system does not give any direct diagnosis and there is the possibility that the samples do not contain enough information to give a clear diagnosis.

The results of the analysis are purely statistical values. Users must reevaluate images and likelihood of the statistical data. Pure interpretation of statistical data is a high risk.

Observations:

- **TissueFAXS** is similar to **TissueFAXSplus** but is for fluorescence samples only and must not be used with brightfield/immunohistochemical samples. **TissueFAXS** consists of the software modules "**TissueFAXS**" and "**TissueQuest**".

- **HistoFAXS** is similar to **TissueFAXSplus** but is for brightfield/immunohistochemical samples only and must not be used with fluorescence samples. **HistoFAXS** consists of the software modules "**TissueFAXS**" and "**HistoQuest**".

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4. In order to use **TissueQuest** application, it is essential for the users to have sufficient knowledge of PC and Microsoft Windows operation system usage.

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1. Introduction

Multicolor immunofluorescence is a major method of cell based research. In order to determine molecular interdependencies it is critically important to measure several markers simultaneously on the same sample (tissue sample or cell culture monolayer).

Over the preceding decades an ever increasing amount of specific molecular markers has been established, helping pathologists, clinicians and researchers to appropriately characterize diseases. For such a characterization, however, a simple “look and conclude” investigation is not sufficient. For many markers, a positive/negative decision does not represent a qualified approach. Rather the question has to be addressed “how much” of a certain marker is present “where” in cells and tissues.

Moreover, measurements of simultaneously determined markers, patterns and functions are required. In order to optimally fit therapies to individual patient's needs, specimens have to be analyzed for an entire set of markers and/or marker combinations in an observer independent manner. In order to meet these requirements, computer aided processing of histological samples is required.

1.1. Purpose

The purpose of this document is to help the user to understand how this application works. It presents **TissueQuest** capabilities and guides the user through the steps required to obtain a good analysis. The document covers the installation of the application, system requirements and main features. In the next section you may read more about the goals of the application and its place in a larger system, so you will understand the purpose and advantages of this application.

1.2. Goals

The **TissueQuest** software is a product for cytometric analysis of tissue sections (“tissue cytometry”), including TMAs and biopsy material, as well as cytological preparations. The initial point of the process chain in tissue analysis using the **TissueQuest** software is importing images to a new project.

Analysis parameters for sophisticated image processing and pattern recognition algorithms have to be optimized for your specific needs.

After optimization of analysis parameters, segmentation is done for all images in the project, which results in a set of numerical data. These data are visualized in scattergrams and are subject to further processing and, finally, statistical analysis.

1.3. Definitions, Acronyms and Abbreviations

Algorithm

A sequence of mathematically realized processing steps applied to an image or part of an image, with the aim to improve image quality and to extract specific information or segment (identify) image objects.

What an algorithm might do:

1. Separate color images;
2. Identify colored regions;
3. Discriminate background from specific cell objects;

4. Recognize individual cells (where does one cell end and neighboring cell begin);
5. Identify cytoplasmatic areas.

Analysis

An analysis is the process of identifying individual nuclei, cells, cellular compartments, and other structures of histological/pathological relevance, respectively. This is accomplished by applying the algorithms of the **TissueQuest** software to the images of tissue sections.

Channel

An image corresponding to a specific marker from a Field of View.

Count (number of events in the respective unit: sample, gate, ROI, quadrant)

Percentage (i.e. how many events are in the respective quadrant region relative to the number of total events) for each of the four quadrant regions.

Cutoff value

A numerical parameter used as a threshold value in a scattergram, in order to distinguish between specific and unspecific staining patterns, allowing determining positive and negative measure events.

Event

A data unit obtained by **TissueQuest** algorithms, representing a quantum of information about a defined object (nucleus, cell).

An event is identified by one or several image processing and pattern recognition algorithms. An event contains information about: area, mean intensity.

Field of View (FOV)

The area of a sample (tissue section) visible in the microscope and captured at one x-/y-/z-position on the slide and with a given optical magnification. An image of one Field of View may contain several markers.

Gate

A subset of events within a scattergram that is selected by defining a region of interest (ROI).

Gates may have any polygonal shape and contain any number of cells. The gates in **TissueQuest** do not support mutual exclusion.

Marker

A molecular labeling component (chemical or biological reagent; e.g. CD3-FITC stain, antibody, genetic probe) that allows the identification of defined cell types, tissue types, cellular or tissue compartments, cellular functions, disease status, etc.

Project

A set of tissue samples which are analyzed together, with individual settings for each channel and sample linking.

Quadrant Statistics

Shows the following values:

- X-Mean (i.e. mean value for the parameter shown on the x-axis);
- Y-Mean (i.e. mean value for the parameter shown on the y-axis).

Scattergram

An x-/y- diagram showing two measurement parameters for all data units or a selection thereof in linear or logarithmic scale.

Within the scattergram, cutoffs can be set in order to define a threshold for each measurement parameter.

1.4. Conventions

In this section you can find listed and explained the conventions used throughout this manual.



- ✓ **Note(s)**: describes important features or instructions.



- ✓ **Tip(s)**: highlights features or hints that can help you save time or avoid difficulties.



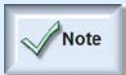
- ✓ **Caution(s)**: warns readers about possible damage to equipment or to data or about potential problems in the outcome of what they are doing.
- ✓ *Italics*: are used in order to highlight a title and also new or unfamiliar terms, usually at their first appearance in the text.
- ✓ **Bold**: are used in order to highlight important terms or to mark the names of the features/GUI elements throughout the text of this manual.
- ✓ →: the arrow indicates a menu choice. For example, choose “**File** → **New**” means you have to choose **New** from the **File** menu.

2. Installing TissueQuest

2.1. Application Dependencies

There are some special requirements concerning the installation of **TissueQuest**. You may check below for the components required to be installed on your computer. It is recommended to have all these components installed before you install the **TissueQuest** product.

Install them in any order, but make sure **TissueQuest** is the last one to be installed.



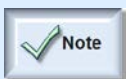
- **TissueQuest** software will not run and operate properly if any of the components is not installed on your **TissueQuest** workstation.
- Installing software programs and *Windows* system components will require *Administrator* privileges on your computer. You might need to ask for support at your IT department if you do not have privileges to install software.

2.2. Windows 3.1 Installer for Windows XP SP2

The Microsoft® Windows® Installer is an application installation and configuration service.

2.3. Microsoft Visual C++ 2005 Redistributables for Windows XP

The Microsoft Visual C++ 2005 Redistributable Package (x86) installs runtime components of Visual C++ Libraries required to TissueQuest on Windows XP (32-bit or 64-bit).



- If you have installed previous pre-release versions of Visual C++ 2005 or Visual Studio 2005, such as Beta 1, Beta 2 or Community Technical Preview (CTP) builds, then you must uninstall these versions via *Add/Remove Programs* in *Control Panel* before installing the final released version.

For further information about this package please visit the *Microsoft* homepage.

2.4. Intel Performance Primitives 5.3

Intel® Integrated Performance Primitives (Intel® IPP) is a software library that increases performance of Intel's latest microprocessors. Incorporating these functions into the **TissueQuest** software will ensure that the potential of your Intel-based PC can be fully exploited for analysis of your samples.

2.5. MARX Dongle Drivers

TissueQuest uses a hardware based security solution from *MARX Software Security*. When purchasing **TissueQuest**, you will also receive an USB dongle that must be inserted in an USB port. In order to successfully use the dongle you must install the corresponding driver for the operating system.

2.6. System Requirements

2.6.1 Hardware Requirements

Minimum

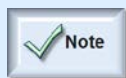
- Intel Pentium 4 Processor - 3 GHz
- 1 GB RAM
- USB-Port (for dongle)
- 100 GB available hard disk space
- Single monitor system with a resolution of 1280x1024

Recommended

- Intel Core2Duo Quad Processor - 3 GHz (or more)
- 4 GB RAM
- Internet connection
- USB-Port (for dongle)
- 1000 GB hard disk space for storing your projects
- Dual monitor system with a resolution of 1680x1050 (or higher)

2.6.2 Software Requirements

- Windows XP
- Windows XP 64-bit
- Windows Vista 32-bit
- Windows Vista 64-bit
- Windows 7 32-bit (Recommended)
- Windows 7 64-bit



- By default, **TissueQuest** needs to be run by a user member of the Administrators group. In this case, all users will share some settings (e.g. institution data, system data, users).

- **TissueQuest** can be also run by a user member of the Users group, in which case every user will have its own settings.

- Please contact your Support team in order to get the desired configuration for your workstation.

Once all the components are successfully installed, the application is ready to be used.

Notice regarding virtual machines

TissueGnostics software is not meant to work on virtual machines. Also, TissueGnostics does not offer support for these configurations, as the software directly uses advanced CPU features and therefore any third layer between the software and the CPU will affect performance and might affect the results of the analysis in an unpredictable way.

3. Login to TissueQuest

After starting the application, a login panel will appear. In order to login to **TissueQuest**, you must enter a username into the **User Name** field and type the proper password into the **Password** field.

Press **Login** to be authenticated.

If the username and password are correct, the login dialog will close and you can start using the application. Otherwise, the application will prompt again for a valid username and password combination.

If you press **Cancel**, the login dialog and the application will close.

The login dialog is used to restrict access to the application. There is one Administrator and any number of users, who may be managed only by Administrator when logged on (for more details please see **Chapter 18**).

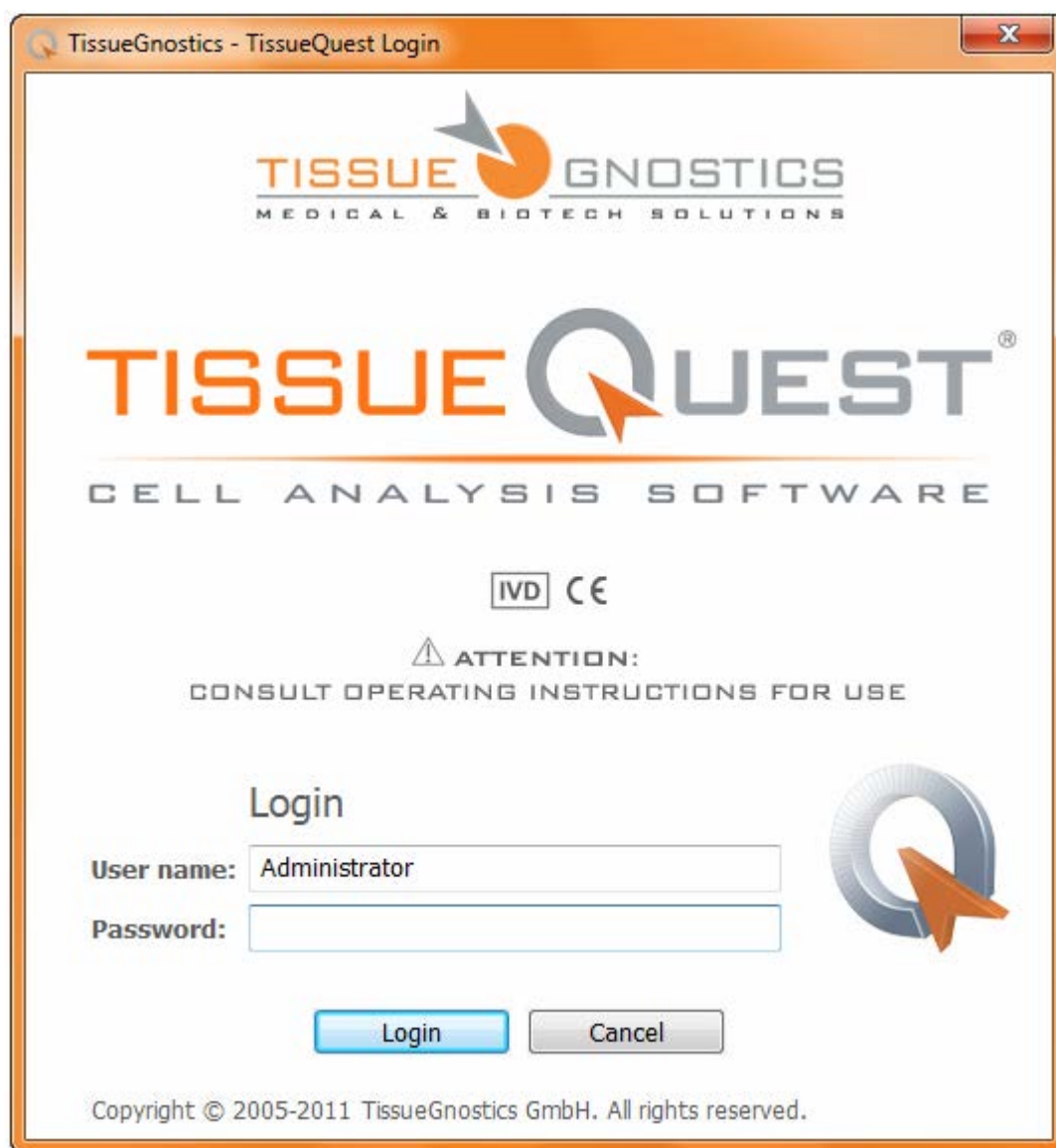


Figure 1 - Login dialog

4. Create new projects

To help you create a new project, **TissueQuest** software offers a five steps wizard that will guide you in a user friendly manner.

Now, take a look at the main toolbar of the application in the picture below. You will notice that only two buttons are enabled: **Create a new project** and **Open an existing project**. The rest of the buttons are disabled until you create or open a project.

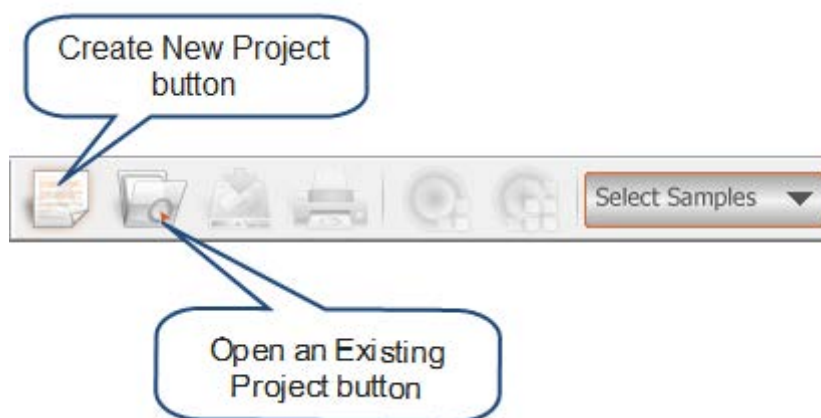



Figure 2 - TissueQuest main toolbar

To start the wizard, press **Create new project** button () from the main toolbar of the application. The first step of the wizard will open.



TIP

- You may also create a new project by going to **File** menu of the application and choose **New...**

4.1. Step 1

Figure 3 - New Project: Step 1

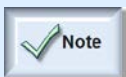
On the first page of the wizard you must enter the project name and the folder where the project will be stored.

What's the name of the project?

Give a name to the project. Automatically, the name of the project will be composed of [**LoginName CurrentDate CurrentTime**]. You can change this name according to your interest.

Where will the project be stored?

Choose a folder where to store the **TissueQuest** projects by using **Browse...** button. In the future, all the new projects will be stored in this folder, unless you will choose another location.



- Please ensure the selected folder exists and that you have enough rights on it, otherwise you won't be able to use it (some types of Windows users have restricted rights).
- Please ensure the selected drive has enough free space available to store your project. Please note that **TissueQuest** will need additional space in order to temporarily store as well as binary mask images.
- **Important:** Avoid storing projects on Desktop, My Documents, or in any other folder located on the system drive (usually C). If the drive is nearly at its full capacity, the system might behave erratically or even stop working if the drive completely runs out

of space. Usually, systems delivered by TissueGnostics have a dedicated storage medium for projects; it is advisable to use it in order to increase TissueGnostics products performance and avoid issues with low disk space. Also in case the system needs to be reinstalled (virus infection, system files being deleted) you might lose data located on the system drive.

After filling in the above fields, press **Next**.

4.2. Step 2

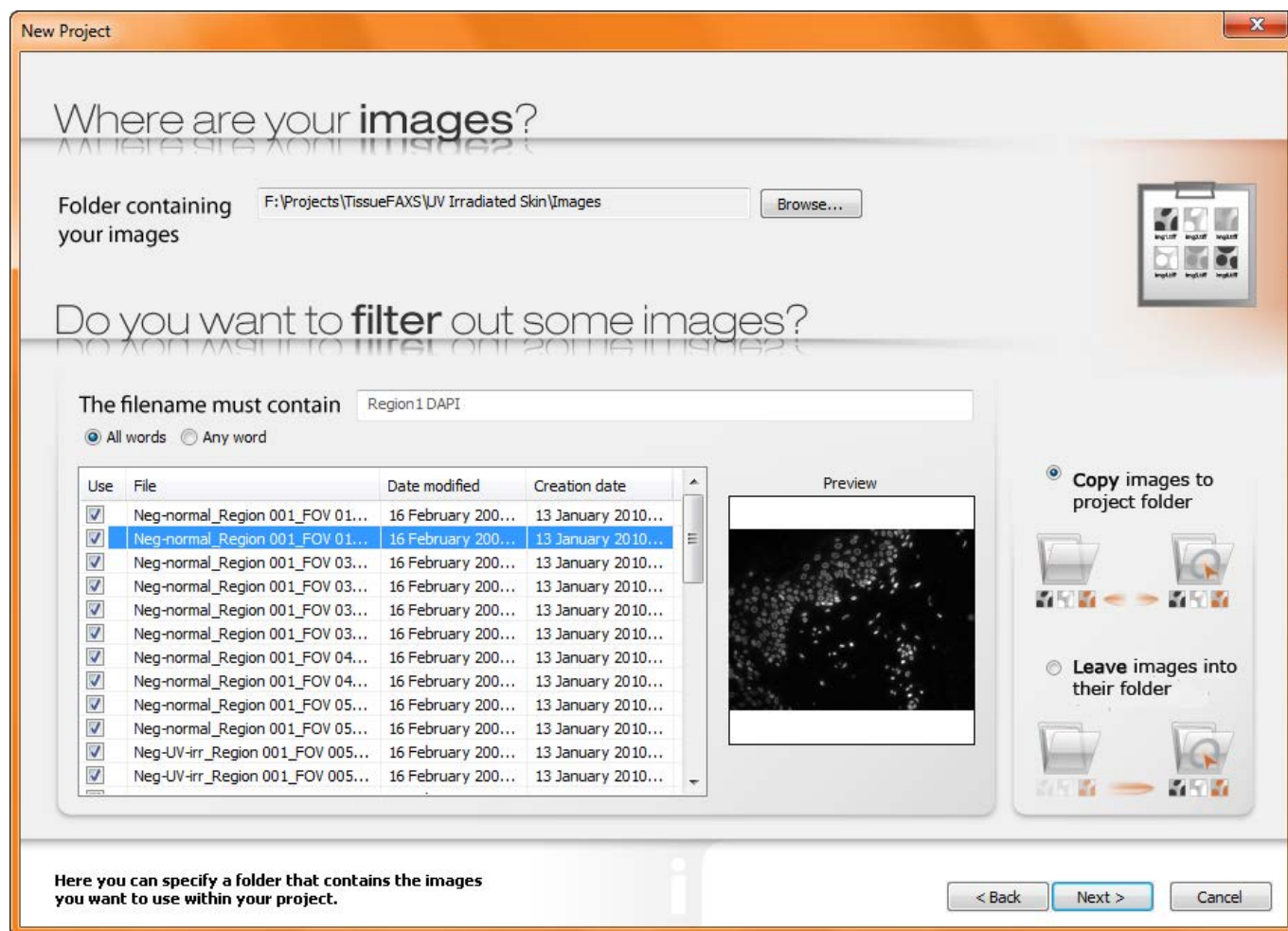


Figure 4 - New Project: Step 2

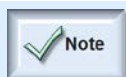
On the second page you must choose the folder that stores the images you want to use in your project. Select the images to be included in the new project by following the next steps:

Where are your images?

Choose the folder that contains the images by using the **Browse...** button.

Do you want to filter out some images?

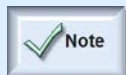
If you wish, you can apply a filter to the names of the files in order to select only those images you need to use in your project. For example, by using the filter "**Region1 DAPI**" and applying the filter as "**All words**", all the files containing the words "**Region1**" and "**DAPI**" will be displayed in the file list.



- An empty character (**Space**) is used as separator between terms. If the search term contains a space like in "**My Sample 1**", the entire term has to be set between quotation marks.

By selecting an image from the list, its content will be displayed in the **Preview** area.

Select a specific type of transfer of images to the project folder ("**copy**" or "**leave**"). By default, the images will be automatically copied into the project folder.



- If the **Leave** option is selected and the original source images (e.g. the **TissueFAXS** project) are removed, then the **TissueQuest** project will lack the source images as well (results will remain, however).

Press **Next** to continue.

4.3. Step 3

What's the **meaning** of the filename?

Mark parts of the filename and explain their meaning by clicking on one of the three buttons below.

Filename example: **Neg-normal** **Region 001** **FOV 014** **20 Rhodamine.tif**

Buttons: ☐ Sample, ☒ Field of View, ☐ Channel

fixed size: ☐ fixed size, ☒ fixed size, ☐ fixed size

Here you can check if all the images were recognized correctly. If some problems appeared you may change the meaning or the problematic filename.

Filename	Sample	Field of View	Channel	Valid
Neg-normal_Region 001_FOV 014_20 R...	Neg-normal	FOV 014	20 Rhodamine	OK
Neg-normal_Region 001_FOV 014_49 D...	Neg-normal	FOV 014	49 DAPI	OK
Neg-normal_Region 001_FOV 032_20 R...	Neg-normal	FOV 032	20 Rhodamine	OK
Neg-normal_Region 001_FOV 032_49 D...	Neg-normal	FOV 032	49 DAPI	OK
Neg-normal_Region 001_FOV 036_20 R...	Neg-normal	FOV 036	20 Rhodamine	OK
Neg-normal_Region 001_FOV 036_49 D...	Neg-normal	FOV 036	49 DAPI	OK
Neg-normal_Region 001_FOV 043_20 R...	Neg-normal	FOV 043	20 Rhodamine	OK
Neg-normal_Region 001_FOV 043_49 D...	Neg-normal	FOV 043	49 DAPI	OK
Neg-normal_Region 001_FOV 054_20 R...	Neg-normal	FOV 054	20 Rhodamine	OK
Neg-normal_Region 001_FOV 054_49 D...	Neg-normal	FOV 054	49 DAPI	OK
Neg-UV-irr_Region 001_FOV 005_20 R...	Neg-UV-irr	FOV 005	20 Rhodamine	OK
Neg-UV-irr_Region 001_FOV 005_49 D...	Neg-UV-irr	FOV 005	49 DAPI	OK

Please mark the parts of the "Filename example".


Buttons: < Back, Next >, Cancel

Figure 5 - New Project: Step 3

TissueQuest needs to know exactly which image file belongs to which **sample**, to which **field of view** within a certain sample and to which **channel** within a certain field of view.

To define the **sample**, the **field of view** and the **channel**, follow the next steps:

What's the meaning of the filename?

By default, the first image file in the list is selected as filename sample. This can be changed, however. To choose the filename to be used as a pattern, right click on any file name in the list and click on **Set a Filename Sample** ().

Mark a part of the **Filename example**, then press the respective button below it (**Sample**, **Field of View**, **Channel**);

For each part of the filename, you may indicate if the part has or has not a fixed size by checking (or unchecking) the **fixed size** checkbox. The **fixed size** checkbox might improve working with filenames where only a part of the name is used to identify the three relevant parts (i.e. sample, field of view, channel) or where one particular part might be characterized by terms of different length in the individual channels (e.g. filter names).

For example, in the image above, the **Channel** doesn't have a **fixed size**. We have 49 DAPI channel (with size of 7) and 20 Rhodamine (with size of 12). In this case, you must uncheck the **fixed size** of the **Channel**.

Press **Next** to continue.

4.4. Step 4

In the next step samples, markers and channels can be associated with each other.

Please note:

The term **sample** refers to a tissue section or cell culture monolayer, which has to be analyzed as one entity and will give one set of results. A sample usually consists of several channels, characterized by the fluorochromes used for labeling (channels usually have the name of the filter or fluorochrome they represent, e.g. "Cy2").

As a fluorochrome can be used with different markers (e.g. antibodies), different samples might have different markers in the same channel (e.g. CD3-Cy2 and CD45-Cy2 – they both have the same channel, Cy2, but two different markers, CD3 and CD45)!

TissueQuest offers to define markers independently of the fluorescence channels used for acquisition and allows assigning specific names to these fluorescence channels. The same name for a channel can be applied to all samples in the project (if the same marker/antibody has been stained on all samples) or you can apply different markers to the same channel in different samples (e.g. if there are four samples stained with CD3-Cy2 and 3 samples stained with CD45-Cy2, the Cy2 channel of samples 1-4 can be named "CD3" while the Cy2 channel of samples 5-7 can be named "CD45").

That means that the corresponding scattergrams will be labeled "CD3" in samples 1 – 4 and "CD45" in samples 5 – 7. If the marker names are not associated to channels, all corresponding scattergrams in samples 1 – 7 will be labeled "Cy2" (and the user has to know what "Cy2" actually stands for in each of the samples).

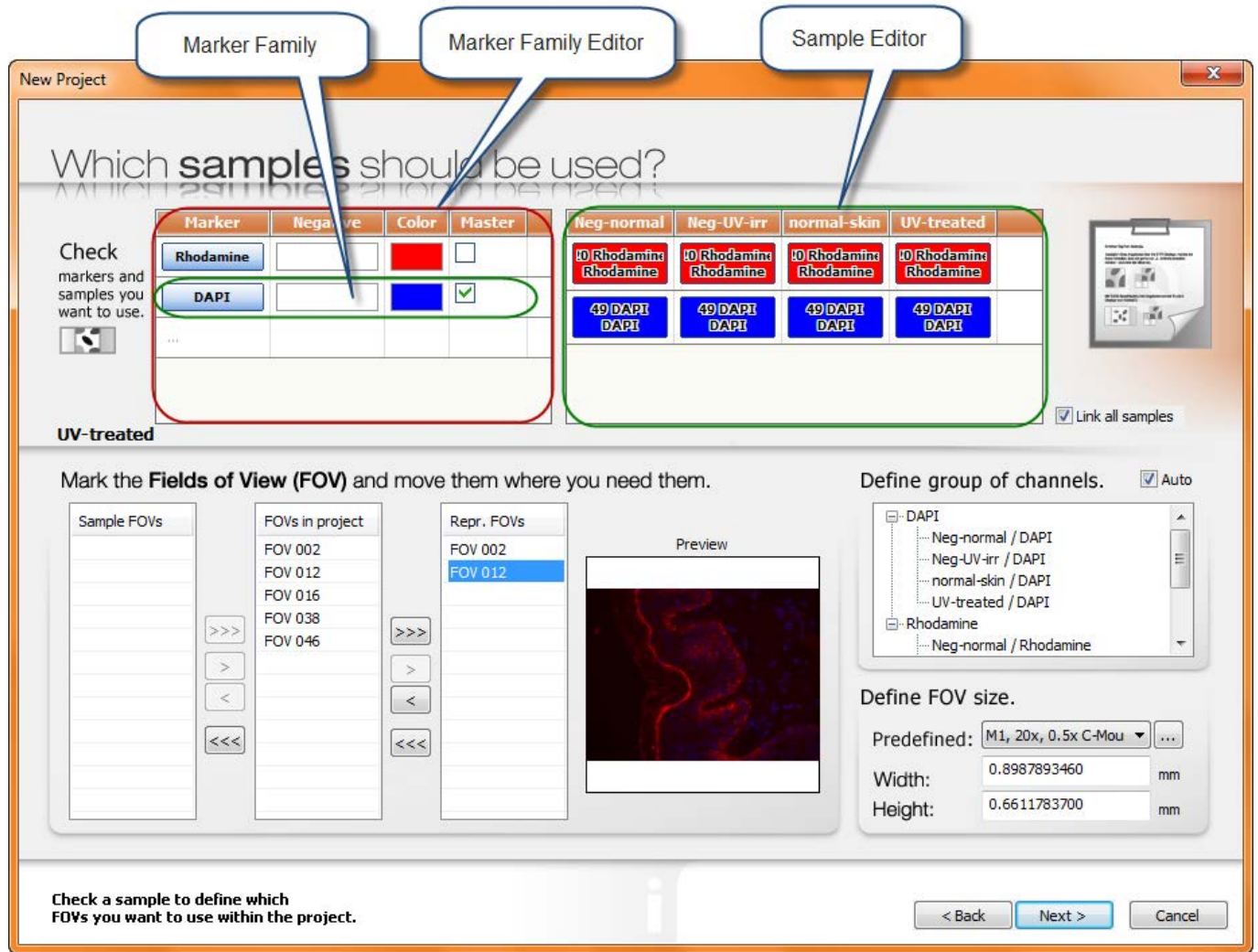


Figure 6 - New Project: Step 4

Page 4 of new Project Wizard contains the following sections:

- Marker family editor
- Sample editor
- Link all samples
- Fields of View
- Group of channels
- FOV size

Marker family editor

In the **Marker Family Editor** you can edit the markers present in the new project. By default, **TissueQuest** creates the markers based on the **Channel** part of the filename, as indicated in **Step 3**. All individual channels in the project have associated unique markers.

The **Marker Family Editor** is organized in lines; each line is referred to as **Marker Family**.

A **Marker Family** contains:

- A marker.
- The negative of the marker, if any is used.
- The color associated with the markers.
- The purpose of the markers: master or non-master channel(s). This determines the channel used for primary cell identification (master channel).

Add new marker

In **Marker Family Editor**, double click on "...". A combo-box will be displayed. Here, you can select a known marker or you can type the name of the new marker (e.g. when a project has different markers for the same channel; compare the example with "CD3-Cy2" and "CD45-Cy2" above). Then, press **Enter**. The newly added marker appears in the **Marker Family Editor**.

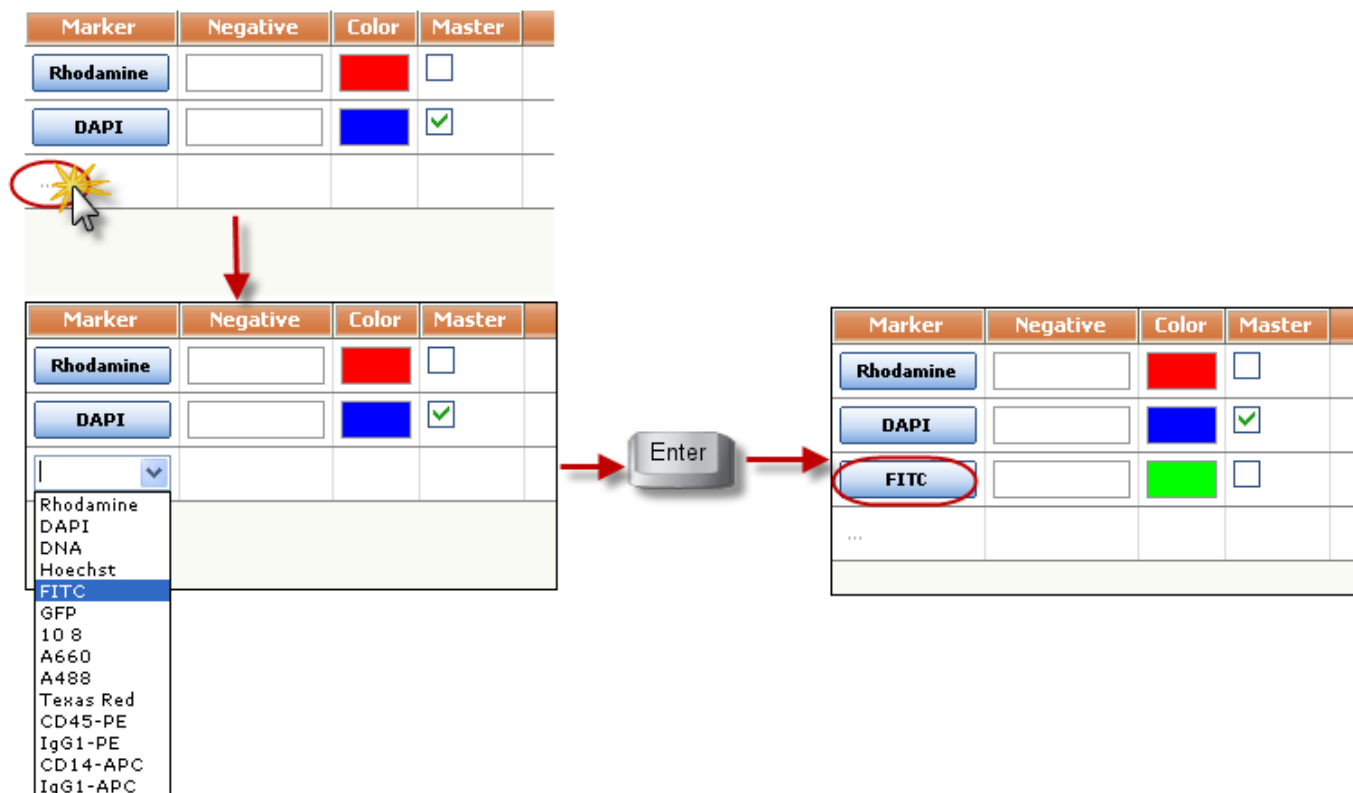


Figure 7 - New Project: Step 4 - Add new marker

If you want to use a marker that is not present in the dropdown list, you can manually add the desired marker by typing its name in the edit box; then press **Enter**.

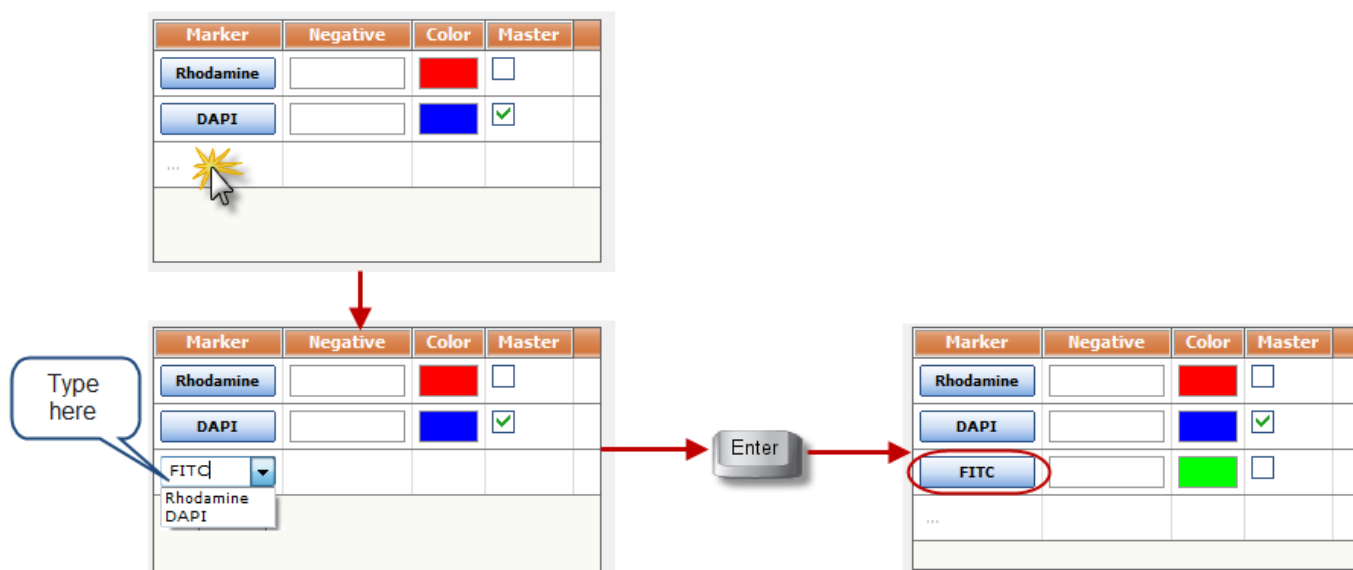


Figure 8 - Manually add new marker

Add negative marker

A negative marker corresponds to a negative control.

A negative control works like this: if the marker sticks on a certain area of tissue from the sample, the negative control will actually stick on the complementary tissue area (that is not covered by the marker).

To insert a negative marker, go to **Marker Family Editor** and double click on the empty rectangle on the right side of the marker. A combo-box will be displayed. Here, you can select a known negative marker or you can type the name of the new negative marker. Then, press **Enter**. The newly added negative marker appears in the **Marker Family Editor**.

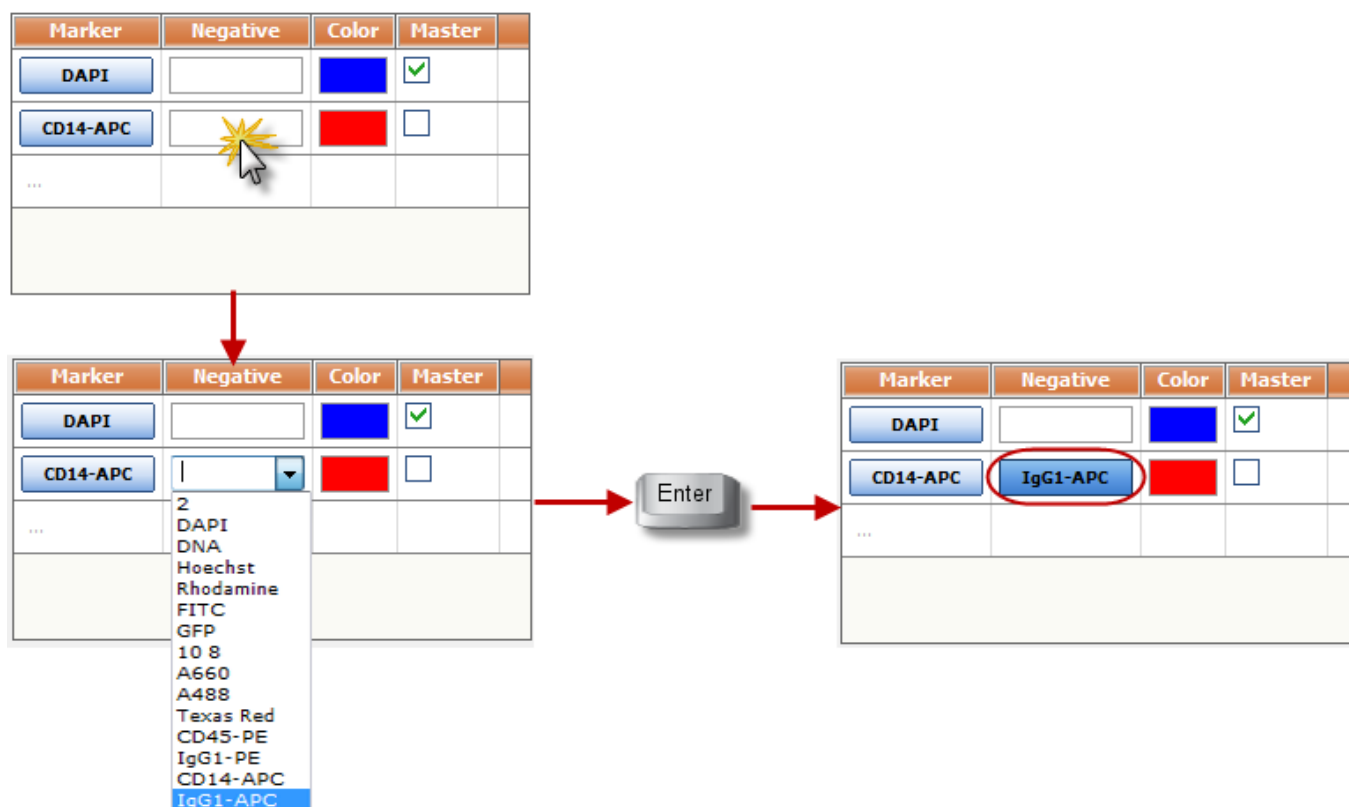


Figure 9 - Add negative marker

If you want to use a marker that is not present in the dropdown list, you can manually add the desired marker by typing its name in the edit box; then press **Enter**.

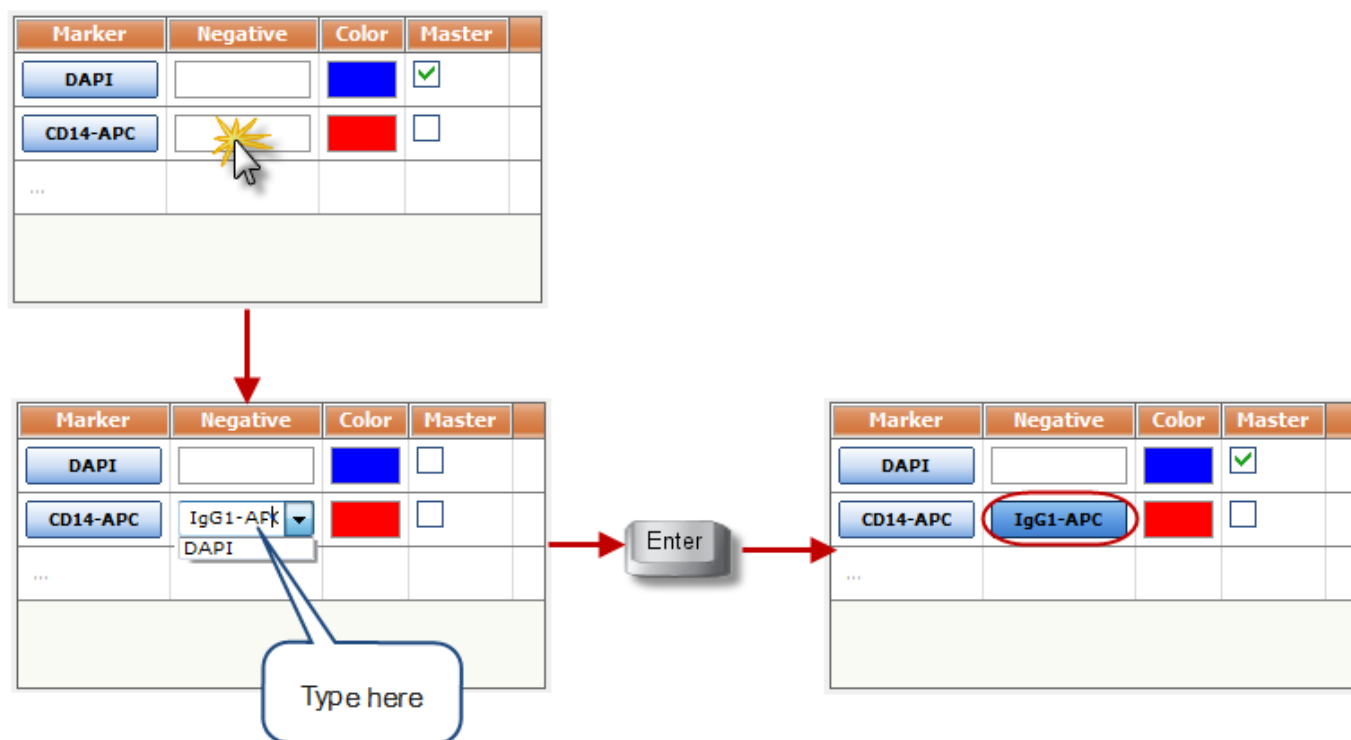


Figure 10 - Manually add negative marker

Benefits of using negative markers

Considering that an experiment has *Sample 1* with the markers DAPI and CD14-APC and *Sample 2* with the markers DAPI and IgG1-APC, the IgG1-APC will be considered as being the negative of CD14-APC.

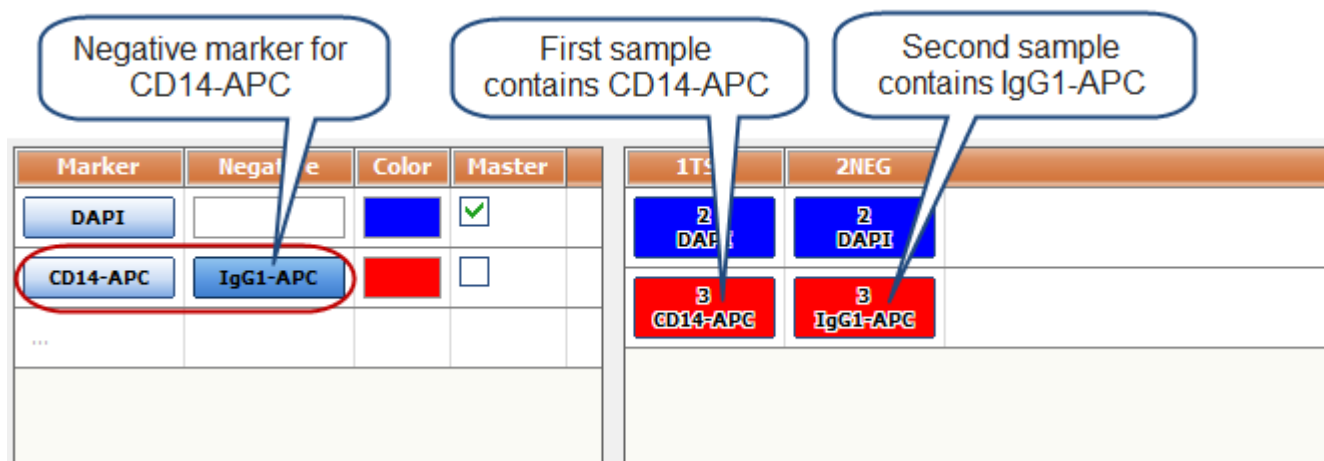


Figure 11 - Using negative markers (a)

If IgG1-APC is set as negative of CD14-APC, the samples can be linked. As a result, the cutoffs and the gates defined on a scattergram are automatically propagated across the linked samples.

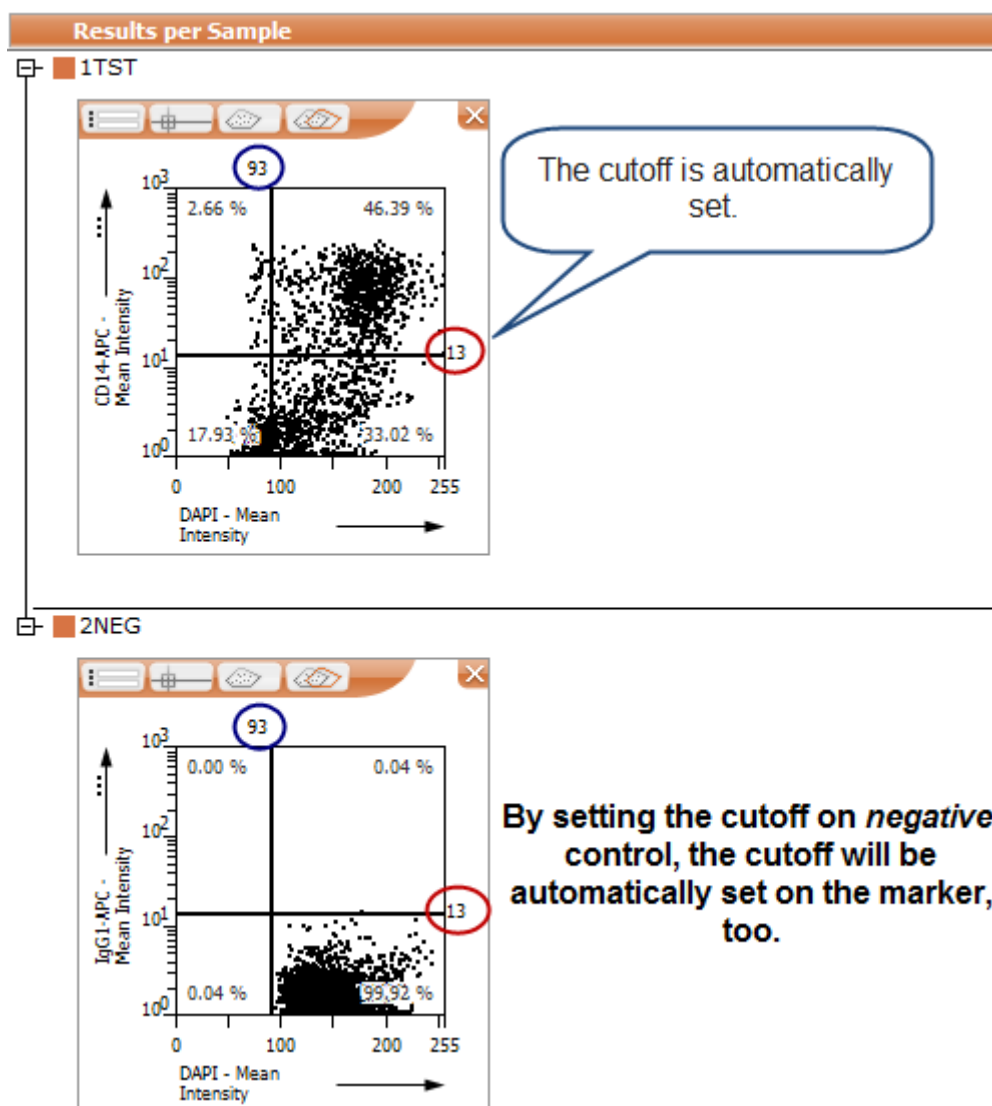


Figure 12 - Using negative markers (b)

If IgG1-APC is not set as negative of CD14-APC, the samples cannot be linked. As a result, the cutoffs and the gates defined on a scattergram are not propagated on the other sample.

In this case, the cutoff and the gates have to be set manually at the desired location.

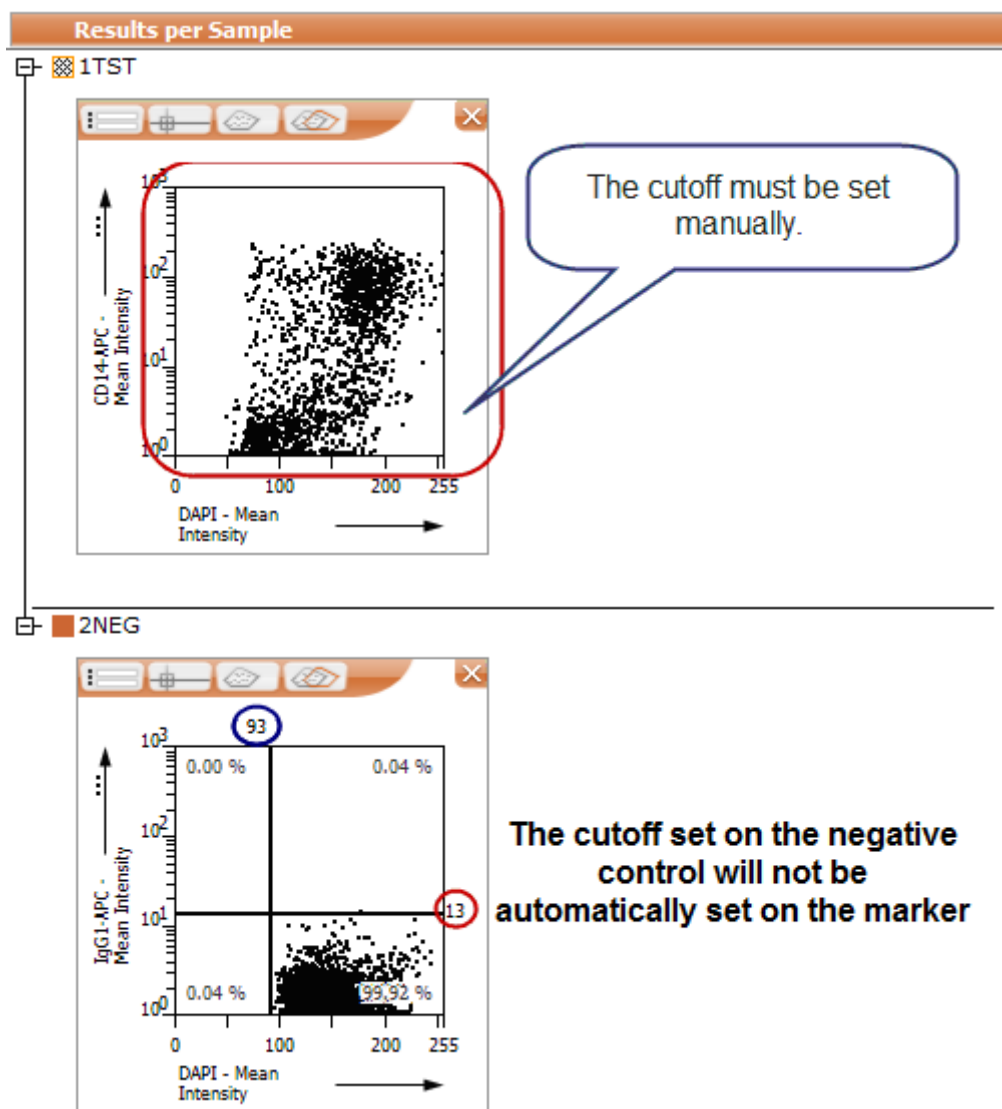


Figure 13 - Using negative markers (c)

Change a marker or Add / Change negative marker

In **Marker Family Editor**, double click on a marker (or its negative). A combo-box will be displayed. Here, you can select a known marker or you can type the name of the new marker. Then, press **Enter**. The newly added marker appears in the **Marker Family Editor**.

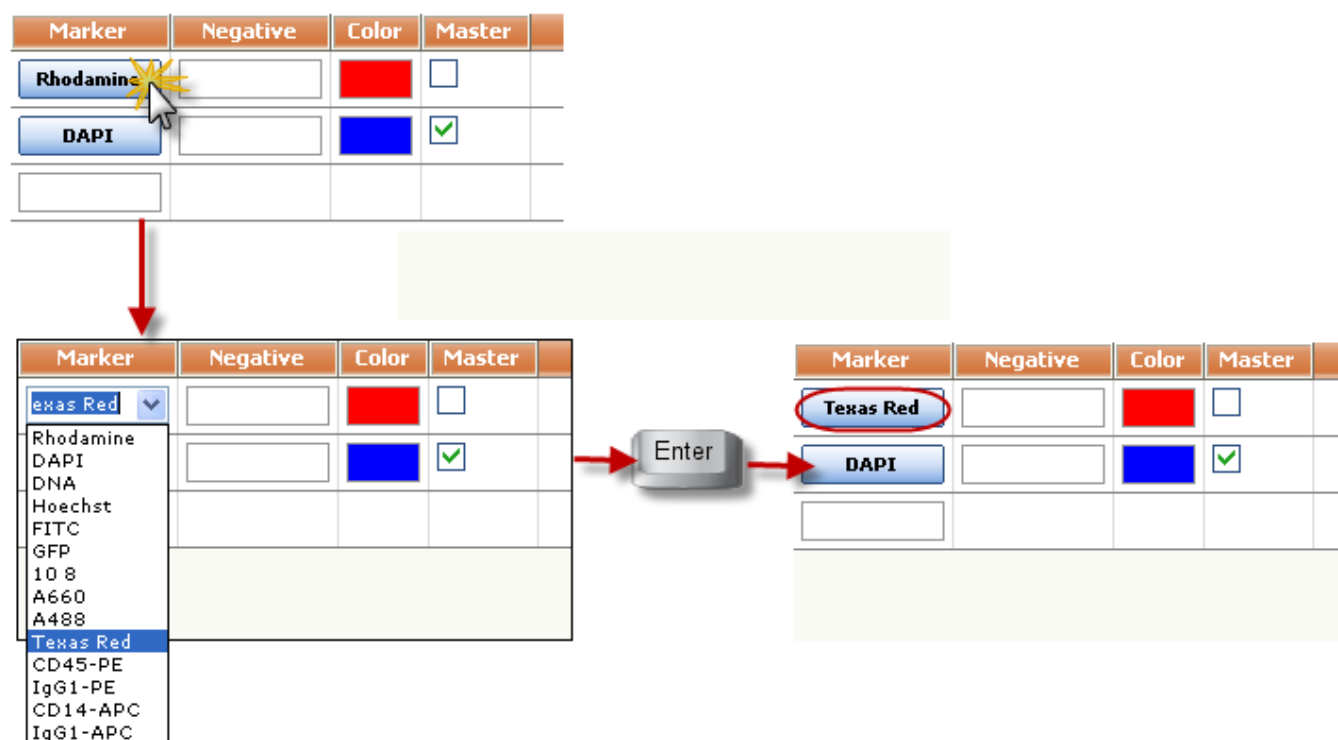


Figure 14 - New Project: Step 4 - Change marker

Change the color of the markers

In **Marker Family Editor**, click on the color corresponding to the markers. A **Color** dialog will be display. Here, select the desired color and then press **OK**.

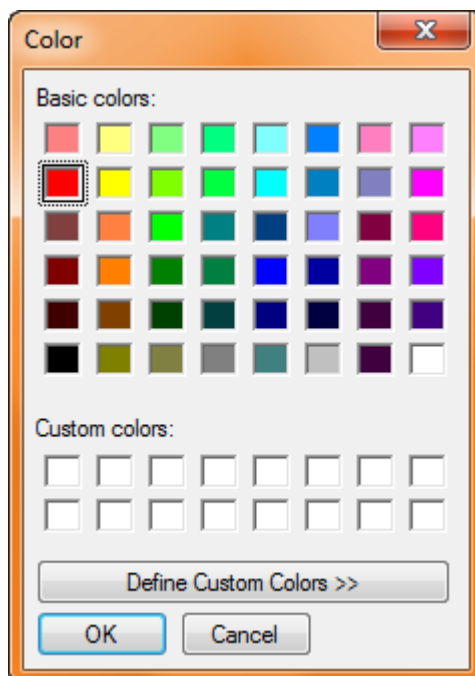


Figure 15 - New Project: Step 4 - Change color

Change the purpose of the markers

In **Marker Family Editor**, use the check-box in the **Master** column in order to change the purpose of the markers.

If the **Master** is checked, then the channels that have these markers associated will become master channels in their samples.

If the **Master** is not checked, then the channels that have these markers associated will not be master channels in their samples (for example, cytoplasmatic channel).

Sample editor

The **Sample editor** offers several functions, which are used to group channels and samples. If two channels are grouped, there will be only one control box shown in the main window of **TissueQuest** to set the segmentation parameters. In other words: if different settings are required for two channels (e.g. a ring mask for the Cy2-channel and a nuclear mask for the Cy3-channel), these two channels **must not be grouped** but have to remain independent.

In the case that different settings of the segmentation parameters are required within the same channel (e.g. the Cy2-channel) in different samples, the user can create groups for certain samples (e.g. group the CD3-Cy2 channel in sample 1 – 4 and create a separate group for the CD45-Cy2 channel in samples 5- 7. That means that the parameters used for analysis of CD3-Cy2 will be the same and the parameters used for analysis of CD45-Cy2 will also be the same, but different from those used to analyze CD3-Cy2.

Associate markers to channels

In **Marker Family Editor**, select a marker by clicking on it. Then, click on a channel in **Sample editor**. You will be asked whether the marker should be associated to all channels with the same name, in the entire project.

- If you choose **Yes**, then the marker will be associated to all channels with the same name.
- If you choose **No**, then the marker will be associated only to the channel on which you clicked.

Use samples

In **Sample Editor**, right click on the name of the sample (in the header). A popup menu will be displayed. Here, you can select to use or not to use the sample. If you choose to not use the sample, then the sample will not be included in the final project.

Use channels

In the **Sample Editor**, select the desired channels (use **CTRL** key for multiple selection) and then right click on them. A popup menu will be displayed. Here, you can select to use or not to use the channels. If you choose to not use the channels, then the channels will not be included in the final project.

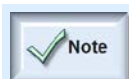
Select sample

In the **Sample Editor**, click on the name of the sample (in the header). The FOVs belonging to the sample will appear in the lists below.

Group channels

Uncheck **Auto Group Channels**. In the **Sample Editor**, select the desired channels (use **CTRL** key for multiple selection) and then right click on them. A popup menu will be displayed. Under the **Group** submenu, select how you want to group the channels.

The parameters for detection algorithm are adjustable individually for each group of channels.



- Master channels and non-master channels cannot be grouped together.

Link all samples

Check **Link all samples** check-box if you want the samples to be automatically linked. See **Chapter 9.2** for more details.

Define group of channels



Auto group channels

Check **Auto** check-box. The channels will be automatically grouped based on their associated marker. All the channels having associated the same marker will be included in the same group of channels.



Rename group of channels

Click twice on the name of the group. A text editor will be displayed. Type the text here and press **Enter** to save the new name. Press **Escape** in order to cancel.

To include some fields of view in the project, follow the next steps:

- Select the desired fields of view in **Sample FOVs** list;
- Press  button next to the right of **Sample FOVs** list to include the selected fields of view in the project;
- Press  button next to the right of **Sample FOVs** list to include all the fields of view in the project.



To make some fields of view representative, follow the next steps:

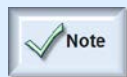
- Select the desired fields of view in **FOVs in project** list;
- Press  button next to the right of **FOVs in project** list to make the selected fields of view representative;
- Press  button next to the right of **FOVs in project** list to make all the fields of view in the project representative.

To remove some fields of view from representative set, follow the next steps:

- Select the desired fields of view in **Repr. FOVs** list;
- Press  button next to the left of **Repr. FOVs** list to remove the selected fields of view from the representative set;
- Press  button next to the left of **Repr. FOVs** list to remove all the fields of view from the representative set.

To remove some fields of view from the project, follow the next steps:

- Select the desired fields of view in **FOVs in project** list;
- Press  button next to the left of **FOVs in project** list to remove the selected fields of view from the project;
- Press  button next to the left of **FOVs in project** list to remove all the fields of view from the project.



- All the fields of view removed from the project will be automatically removed from the representative set.

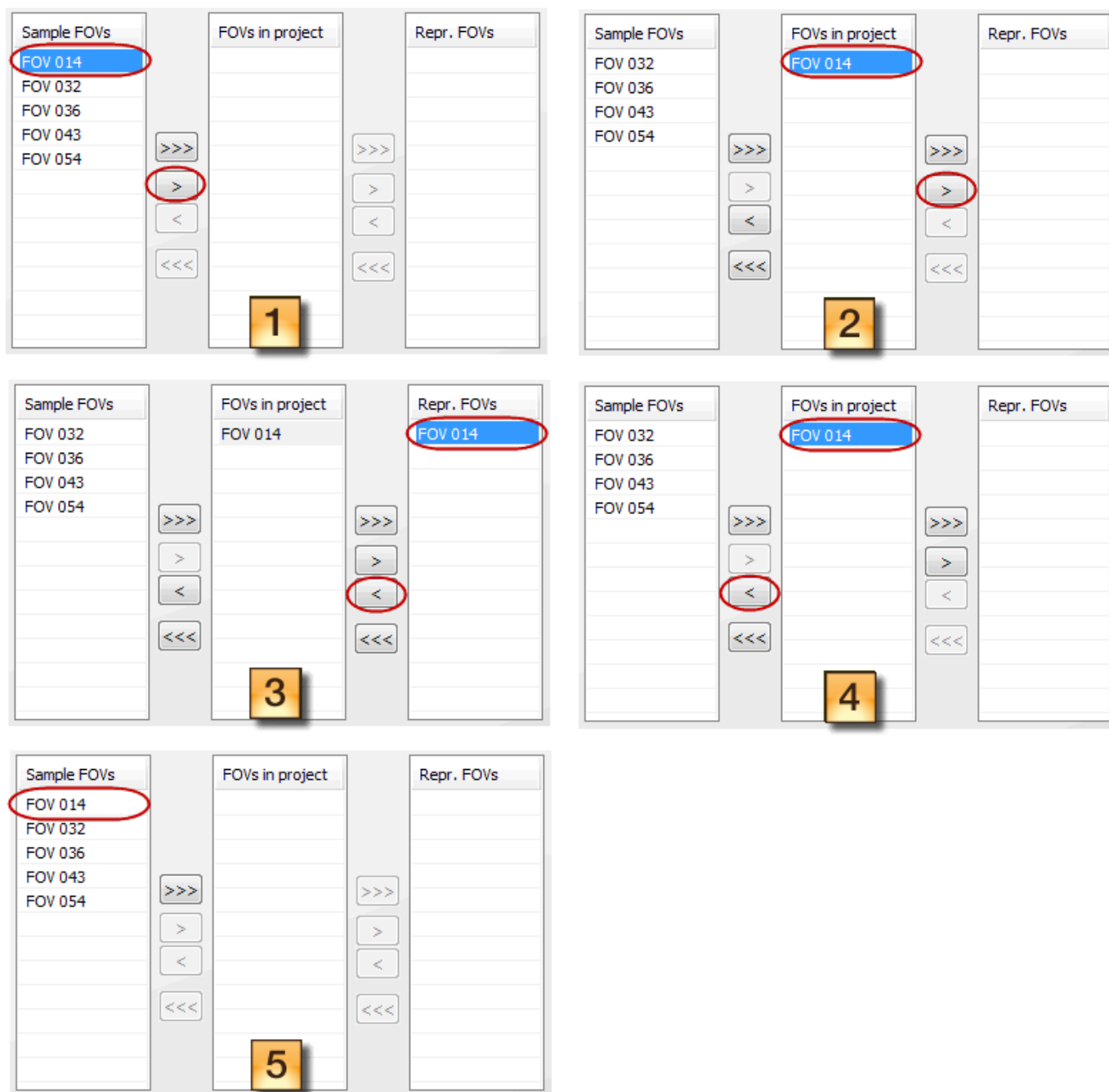


Figure 16 - Include/exclude one FOV from project/representative list

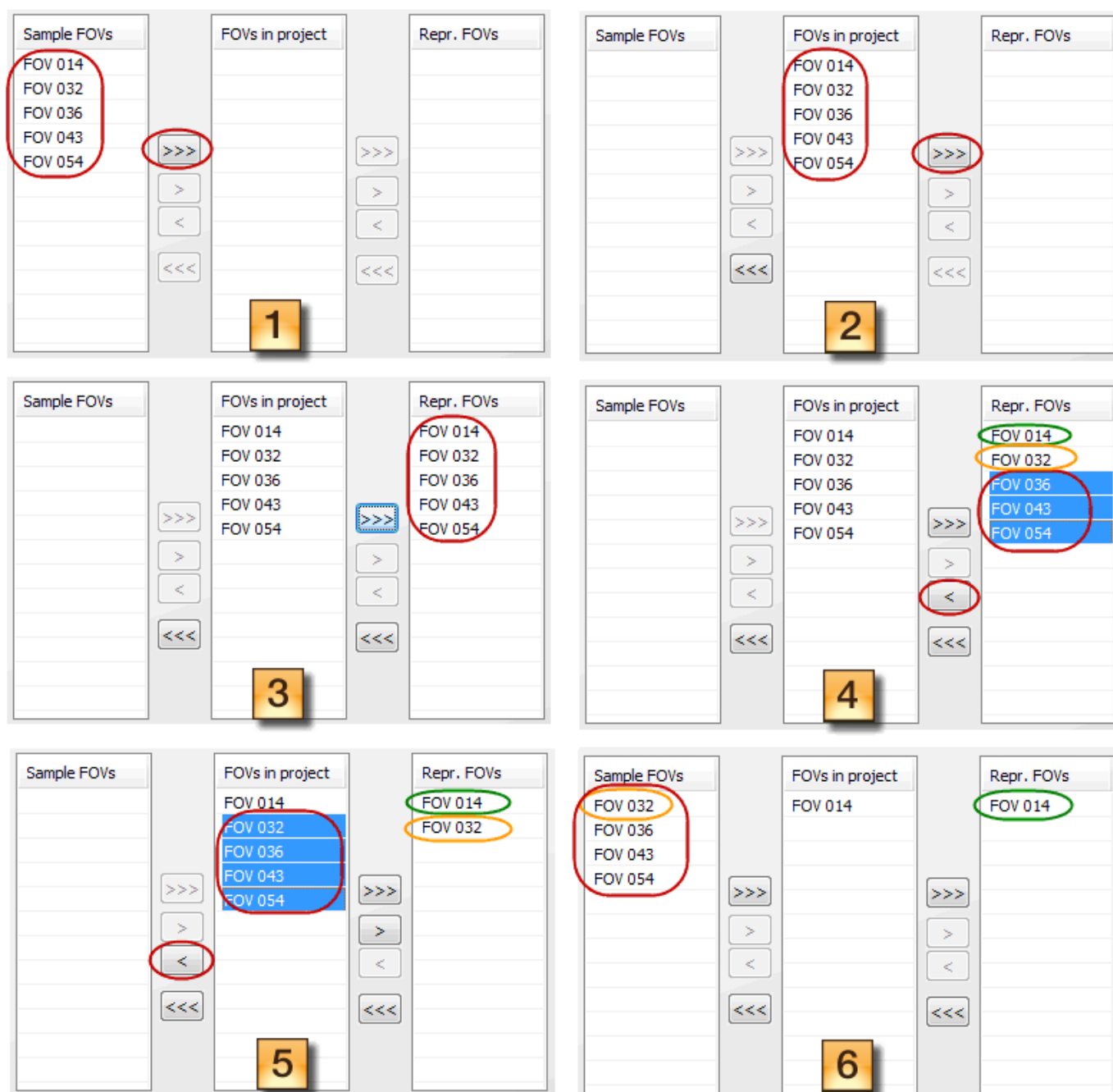


Figure 17 - Include/exclude all FOVs from project/representative list

FOV size**Select predefined template**

Select a predefined template for FOV' size from the **Predefined** combo-box.

Manage templates

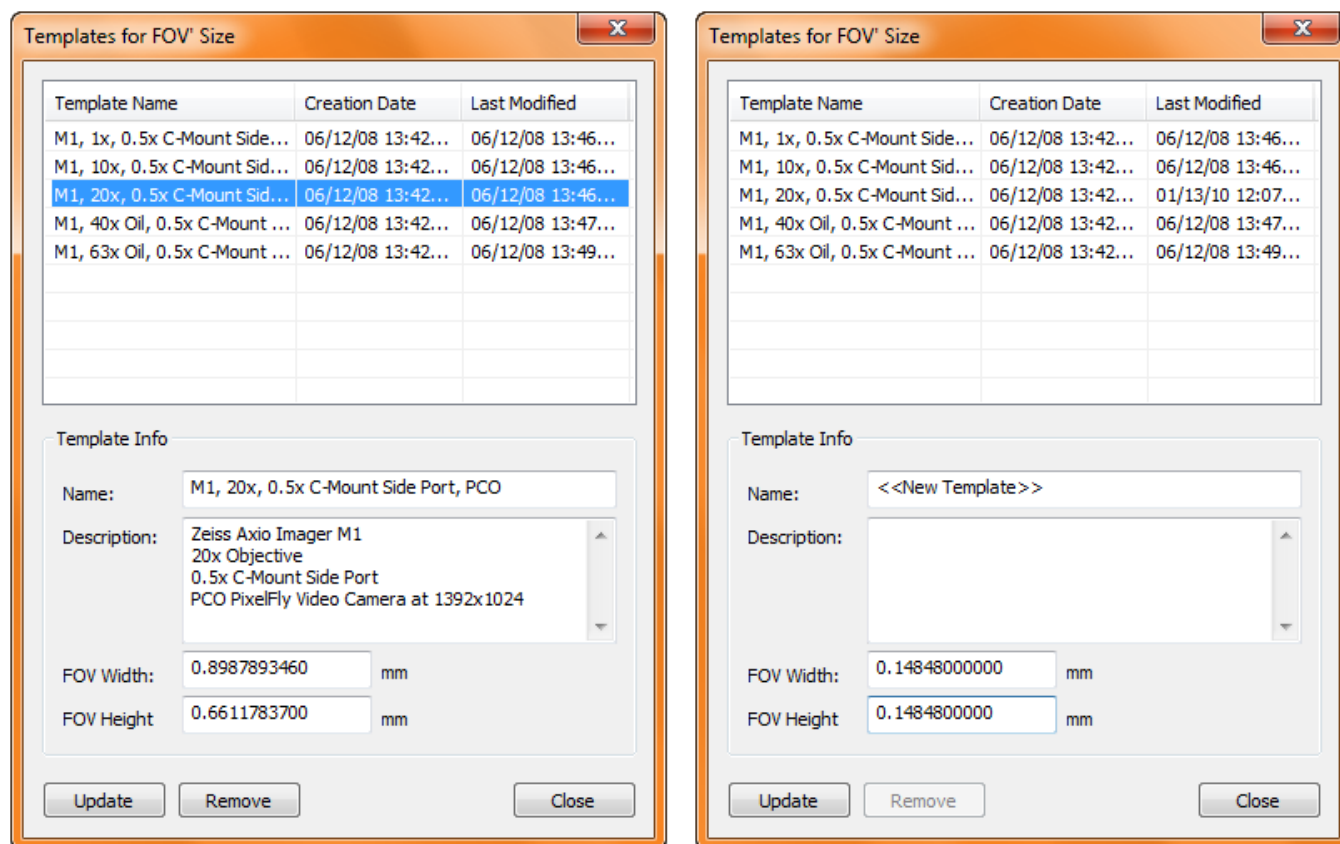
To manage the templates for FOV size, use "..." button next to the right of the **Predefined** combo-box. A dialog will appear in which you can manage the templates for FOV' size. For more details, see [Chapter 4.4.1](#).

Manually specify the size

You can manually specify the size of the FOV by writing its width and height in the **Width** and **Height** edit-boxes.

4.4.1 Templates for the FOV' size (scaling)

In this dialog box, you can define new templates in order to adjust the size/scaling of the fields of view. You can also change or remove an existing template.



See or edit details of a template

Create new template

Figure 18 - Templates for FOV' size

To create a new template, follow the next steps:

- Enter the name and the description of the template. Then, enter the size of the field of view;
- Press **Update** button to create and store the template;
- If a template with the same name already exists, that template will be updated. A new template will not be created.

To modify an existing template, follow the next steps:

- Select a template from the list;
- Change the description of the template and the size of the fields of view;
- Press **Update** to store new information;
- If you also change the name of the template, a new template will be created. The old template will not be updated.

To remove an existing template, follow the next steps:

- Select the template from the list;
- Press **Remove** button.

4.5. Step 5

New Project

Here is the **summary** of your settings

On the right you have a summary of the settings you defined in the previous steps.

This is an overview to check if everything is set as you like.

Press „Back“ to change previous settings or „Finish“ to accept the current project settings.

Project name: **UV Irradiated Skin**

Basics

Cell identification strategy: Nuclear

Storage

Project is stored in: F:\Projects\TissueQuest\UV Irradiated Skin

Your images in the folder ...: F:\Projects\TissueFAXS\UV Irradiated Skin\Images

... will be copied to: F:\Projects\TissueQuest\UV Irradiated Skin\Images

Samples

Neg-normal	Channel	Marker	Master
	20 Rhodamine	Rhodamine	
	49 DAPI	DAPI	*

This is the overview of the settings you defined before.
If everything is ok, press Finish.

< Back Finish Cancel


Figure 19 - New Project: Step 5

This overview allows the user to check up if the settings are correct.

Press **Back** to change previous settings or **Finish** to accept the current project settings and create the project.

5. Open Existing Project



To open an already existing project, press **Open an existing project** button () from the main toolbar of the application. In the dialog that appears, browse for the desired project and then press **Open**.

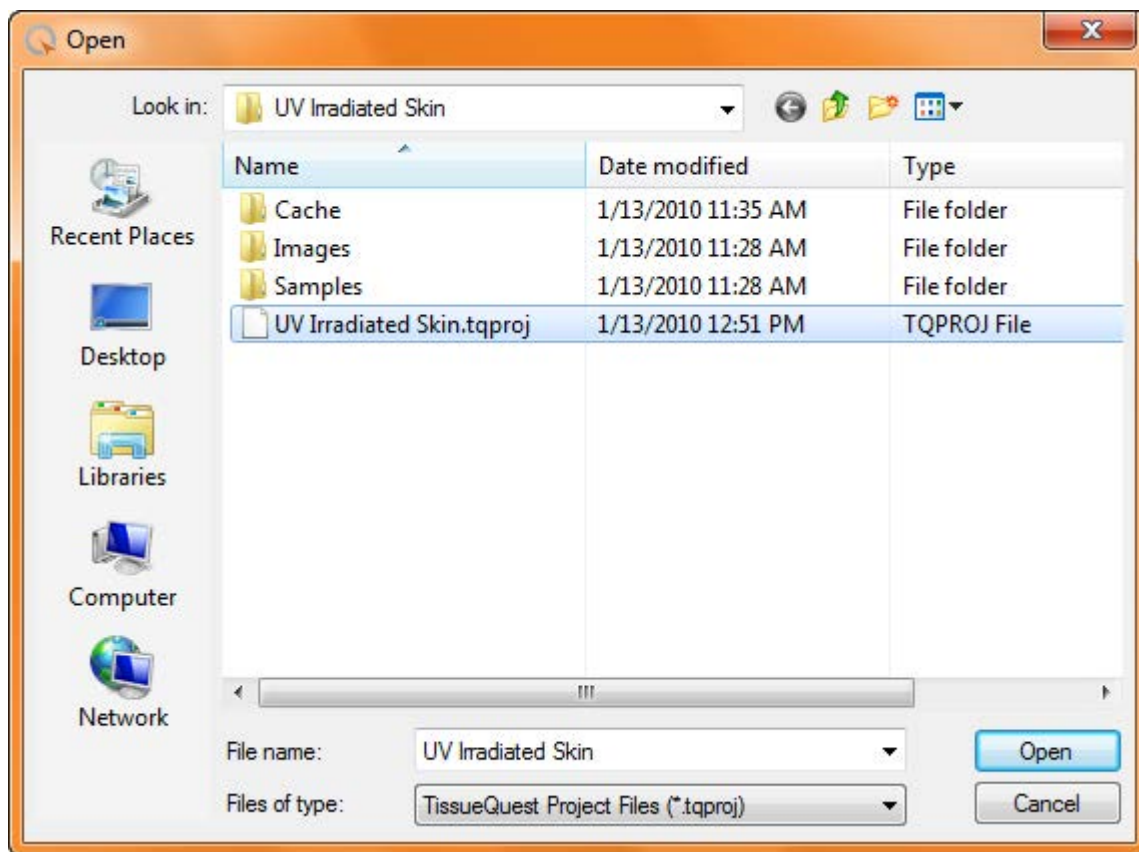


Figure 20 - Open an existing project



- You may also open a project by going to **File** menu of the application and choose **Open...**

5.1. Save Project



To save the project press **Save current project** button (). The project will be saved.



- You may also save a project by going to **File** menu of the application and choose **Save Experiment...**

5.2. Print Project



To print the project press **Print current project** button (). For more details please see **Chapter 14.1.**

6. Project Menu

To access the **Project** menu, go to **TissueQuest** menu bar and choose **Project**. The following menu will appear:

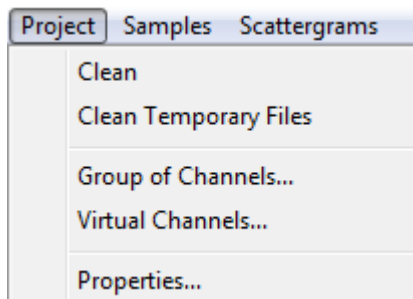


Figure 21 - Project menu

6.1. Clean

Choose **Clean** option if you want to remove from your project all results (detected events) and temporary images (mask and overlay images). A warning message like the one below will appear:

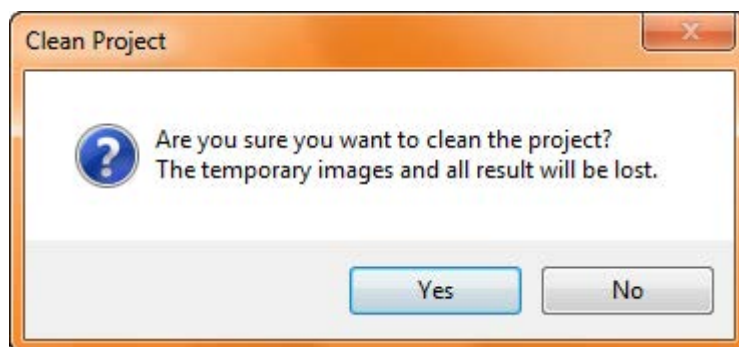


Figure 22 - Clean Project warning message

6.2. Clean Temporary Files

Choose **Clean Temporary Files** option if you want to remove from your project only temporary images (mask and overlay images). This helps to save space on your hard disk, especially for archiving **TissueQuest** projects. (**Note:** by re-running the analysis, all the temporary files can be created again at any time in the future; data and scatterplots remain unchanged as long as the settings for analysis remain unchanged). A warning message like the one below will appear:

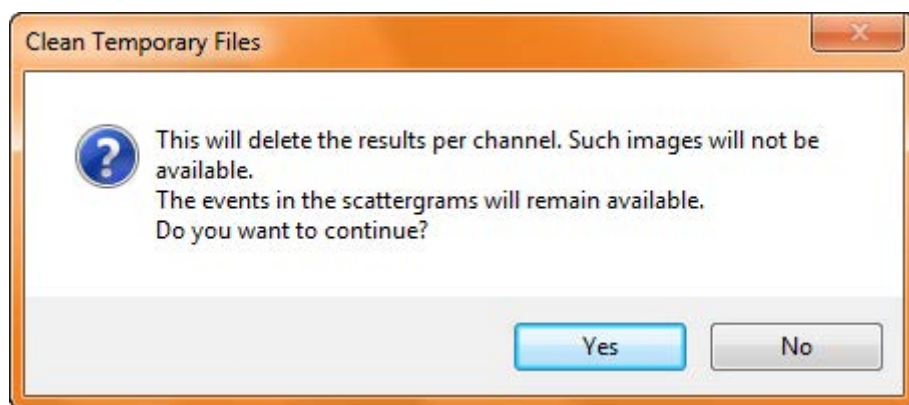


Figure 23 - Clean Temporary Files warning message

6.3. Group of Channels

By choosing **Group of Channels...** from the **Project** menu, the **Group of Channels** dialog will appear:

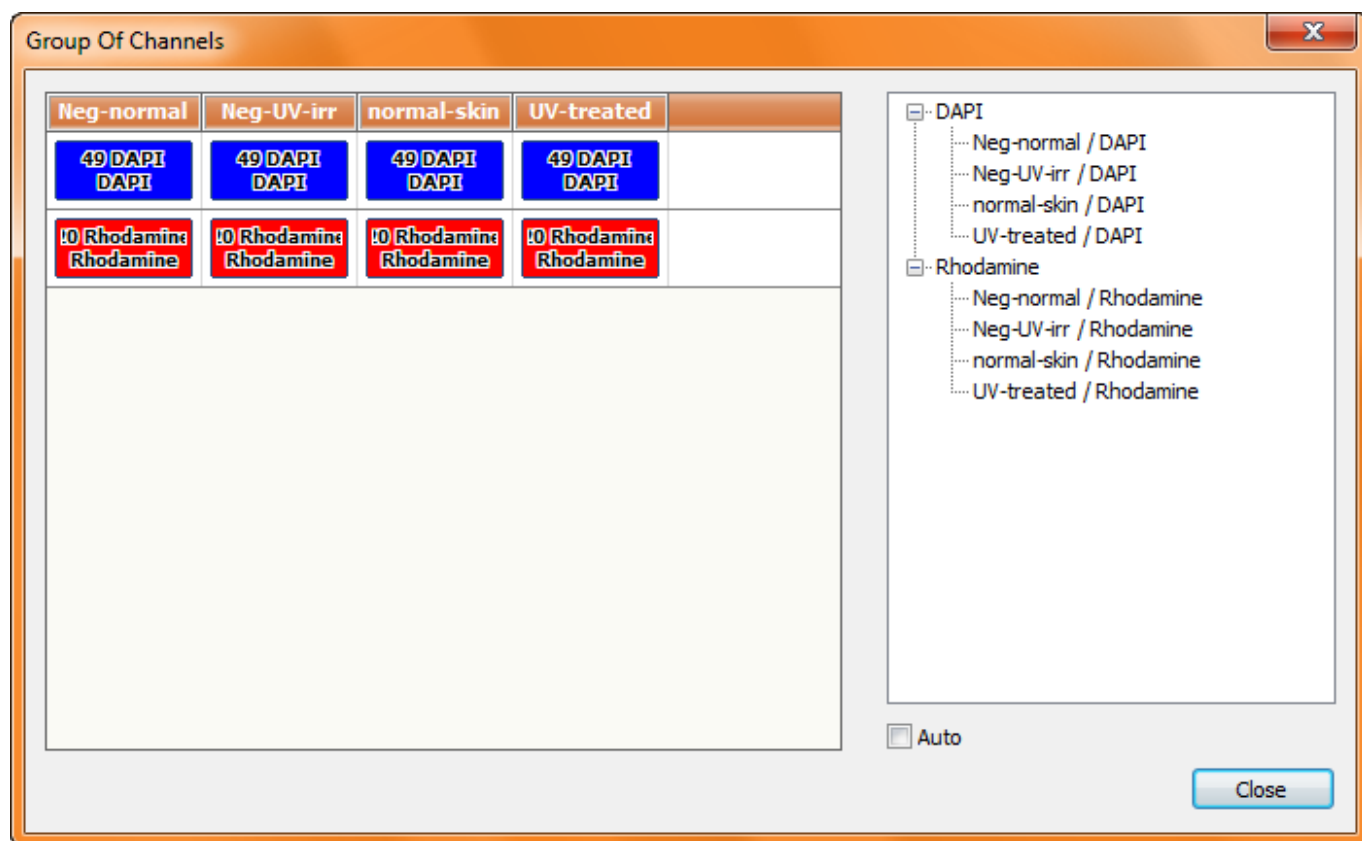


Figure 24 - Group of Channels dialog

Grouping channels means including in a group more channels that share same parameters.

Auto group channels

You can group channels automatically, by checking **Auto** checkbox. The channels will be automatically grouped based on their associated marker. All the channels having associated the same marker will be included in the same group of channels.

Manually group channels

To start grouping channels yourself, uncheck **Auto Group Channels**. In the **Sample Editor**, select the desired channels by clicking on each channel (hold pressed **Ctrl** key for multiple selection) and then right click on them. A popup menu will be displayed. Under the **Group** submenu, select how you want to group the channels:

- **All channels with [Name] marker:** this option will include in the group all channels containing a certain marker.
- **Only selected channel:** this option will include in the group only the channels you have selected.

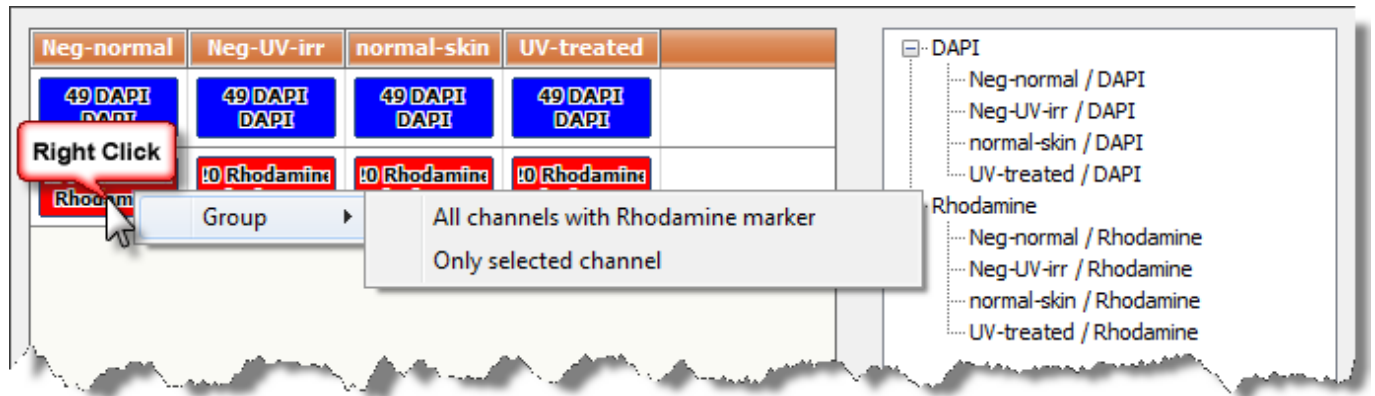


Figure 25 - Group of Channels dialog: grouping channels



TIP

- The parameters for detection algorithm are adjustable individually for each group of channels.
- You cannot group master and non-master channels.

Rename group of channels

Click twice on the name of the group. A text editor will be displayed. Type the text here and press **Enter** to save the new name. Press **Escape** in order to cancel.

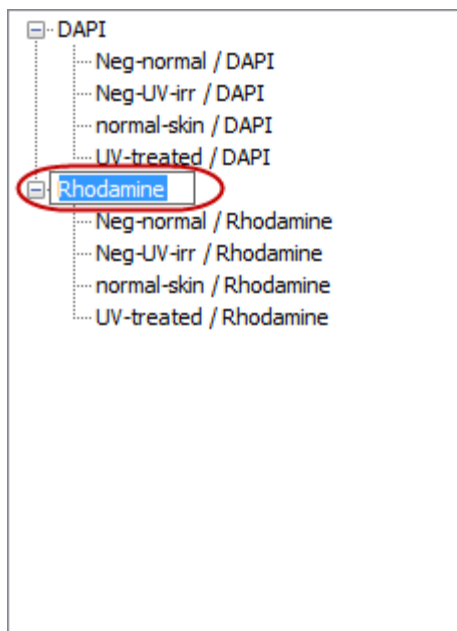


Figure 26 - Rename group of channels

6.4. Virtual Channels

By choosing **Virtual Channels...** from the **Project** menu, the **Virtual Channels** dialog will appear:

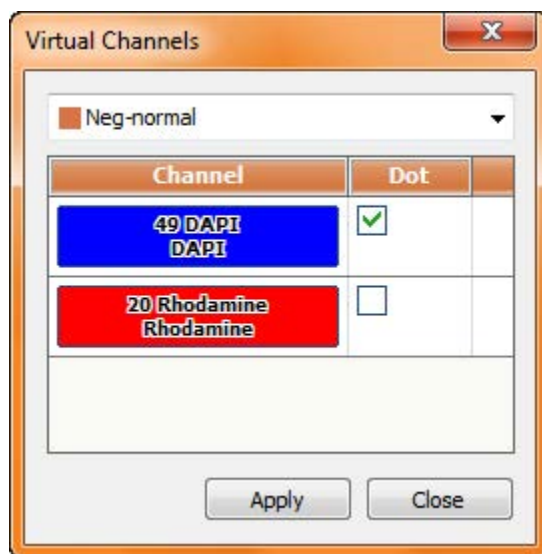


Figure 27 - Virtual Channels dialog

Here, you can choose the sample and the channel in order to apply **DOT finder detection** (please see [Chapter 15.3](#)).

Press the  button to access the dropdown menu and to choose the desired sample.

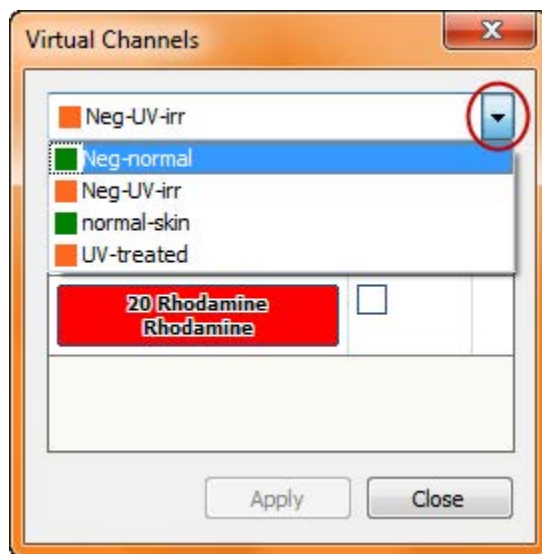


Figure 28 - Virtual Channels dialog: dropdown list

For each original channel, you can mark the checkbox from the **Dot** column in order to create a virtual channel on which to measure the dots.

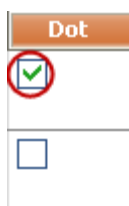


Figure 29 - Virtual Channels dialog: mark Dot checkbox

Press **Apply** button to apply the changes.

A new virtual channel is created; it shares the original image with its original channel.

6.5. Individual Dots Settings

Same Settings for all DOT Channels

If you want to have the same settings for all DOT channels, you have to go to **Project** from the menu of the application, and then choose the **Virtual Channels** option.

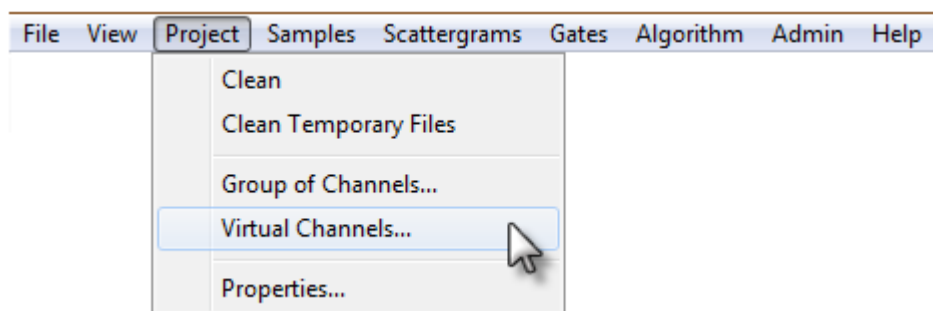


Figure 30 – Project Menu – Virtual Channels option

A dialog box will appear where the user can select all desired DOT channels (please see image below).

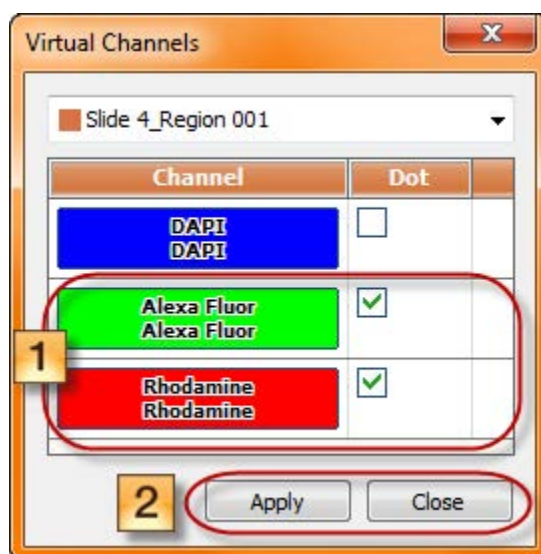


Figure 31 – Virtual Channels: selecting DOTs channels

After selecting the desired DOT channels you have to press the **Apply** and then the **Close** buttons. In the application at the **Parameters** section, the selection will look like this:

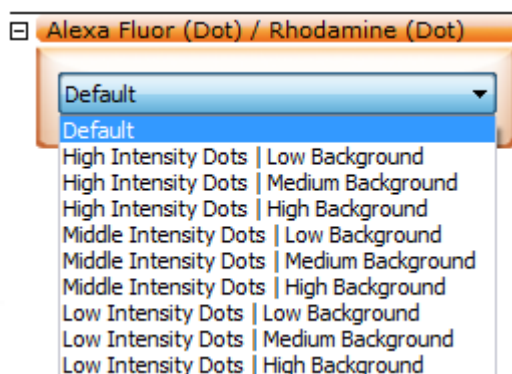


Figure 32 – DOT settings dropdown box

You can select now the desired settings in order to get the right DOTS detection.

Different Settings for Individual DOT Channels

If you want to have separate settings for **individual DOT channels**, you have to go to **Project** from the menu of the application, and then choose **Virtual Channels** option.

In the dialog box that appears you will have to select one of the DOTS channels to be introduced. After the selection is made, press the **Apply** and then the **Close** buttons (see image below).

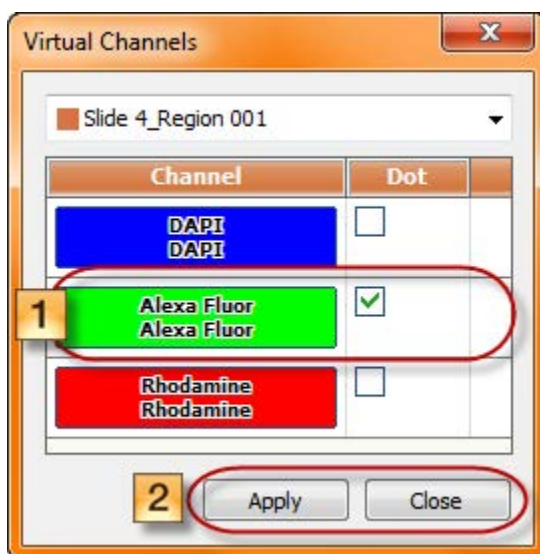


Figure 33 – Virtual Channels: selecting DOT channel

In order to introduce the second DOT channel, you have to go again to **Project** from the file menu of the application and choose the **Virtual Channels** option.

In the dialog box that appears you will have to select the second DOT channel that you want to introduce. After the selection is made, press **Apply** and then **Close** buttons (please see image below).

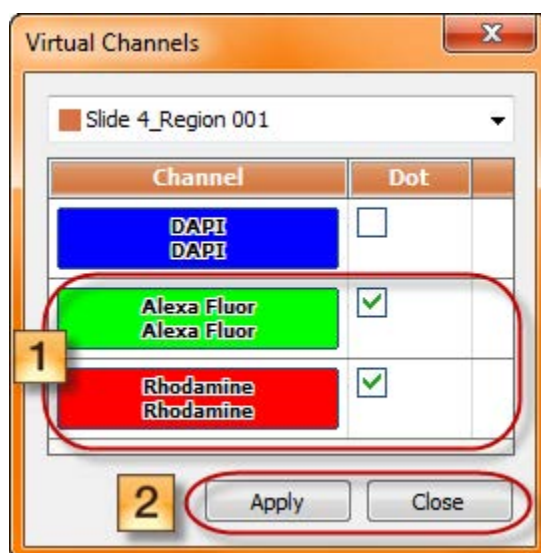


Figure 34 – Virtual Channels: selecting DOTS channels

After performing these operations, there will be two DOTS markers in the application at the **Parameters** section (see image below).

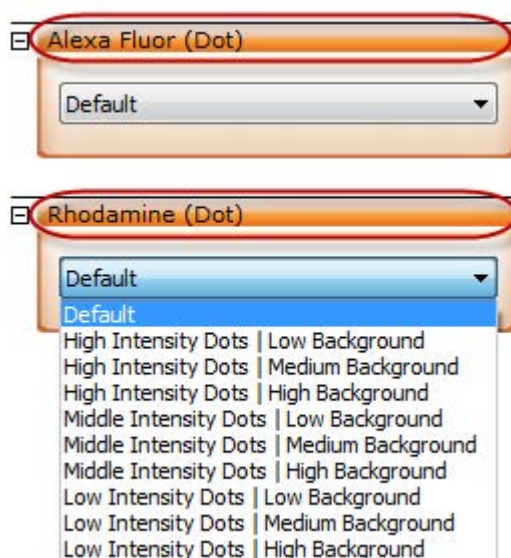
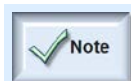


Figure 35 – DOT settings dropdown box



- You can have different settings for detection for each DOT marker.

6.6. Properties

For details concerning **Properties** option, please see [Chapter 12](#).

7. Main Window

7.1. Components of the Main Window

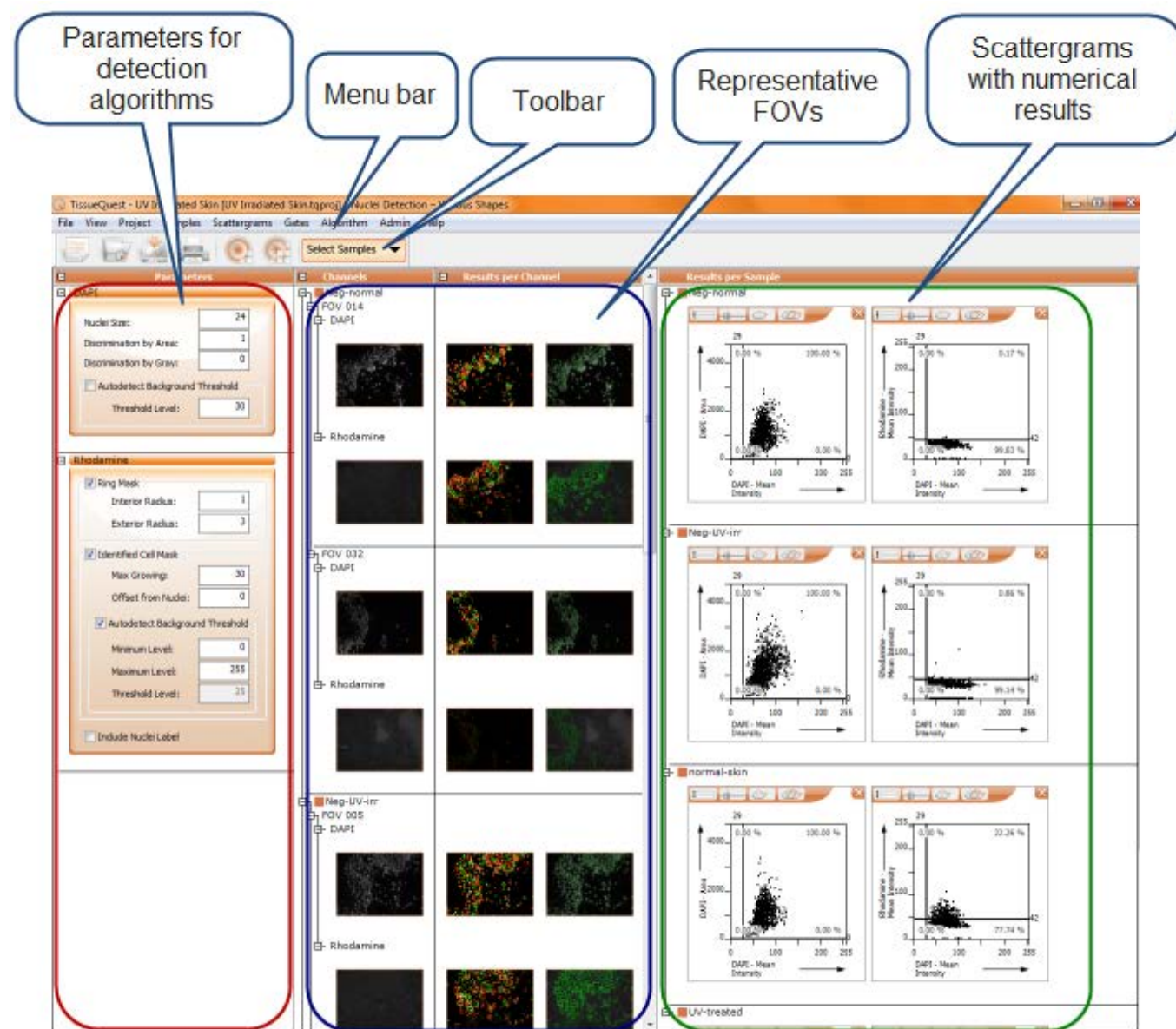


Figure 36 - TissueQuest: Main window

The **main window** of the application (the window that is the focus of the user's actions) consists of five parts, as seen in the picture above:

- the menu bar;
- the tool bar;
- on the left, you can adjust the parameters for the detection algorithms;
- displayed in the middle there are the representative fields of view;
- displayed on the right there are the scattergrams.

7.2. Menu

In this section you can get familiar with the **TissueQuest** menu bar in a short glimpse. You can find explained all the menus graphically presented below in the corresponding sections of this manual (please follow given references).

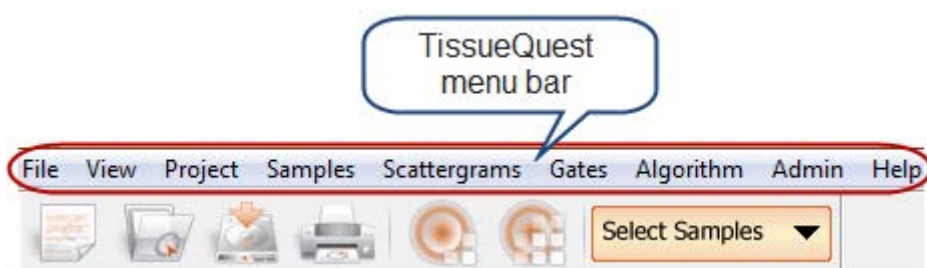


Figure 37 - TissueQuest: Menu

File

See : [Chapter 5](#), [Chapter 14](#).

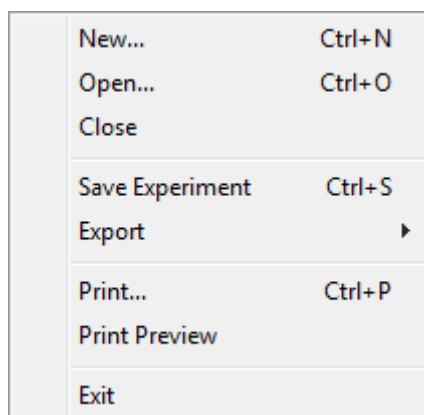


Figure 38 - File menu

View

Check any of the listed elements from this menu to have it displayed.

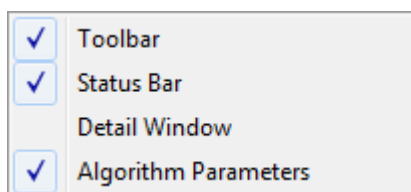


Figure 39 - View menu

Project

See: [Chapter 6](#), [Chapter 7.5](#), [Chapter 12](#).

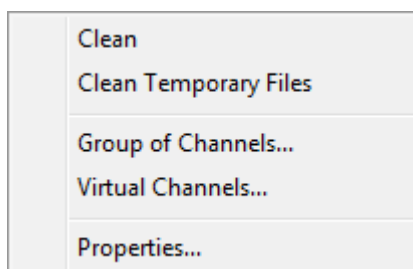


Figure 40 - Project menu

Samples

See: **Chapter 9.**

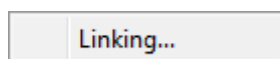


Figure 41 - Samples menu

Scattergrams

See: **Chapter 10.**

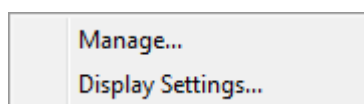


Figure 42 - Scattergrams menu

Gates

See: **Chapter 10.5.**

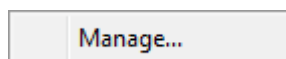


Figure 43 - Gates menu

Algorithm

See: **Chapter 16.**

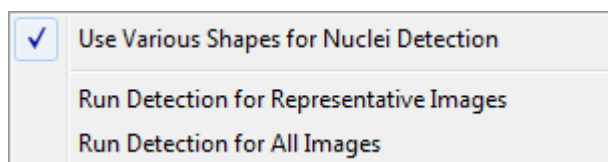


Figure 44 - Algorithm menu

Admin

See: **Chapter 18.**

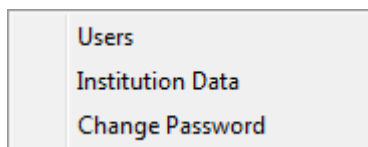


Figure 45 - Admin menu

Help

See: **Chapter 19**.

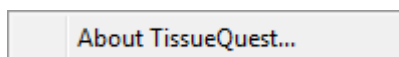


Figure 46 - Help menu

7.3. Toolbar

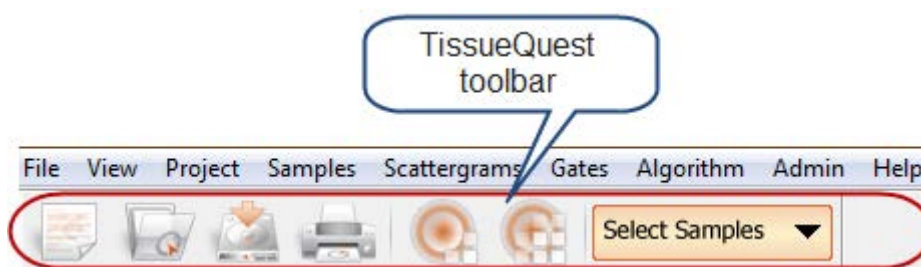


Figure 47 - TissueQuest: Toolbar



Create a new project (see **Chapter 4**)



Open an existing project (see **Chapter 5**)



Save current project (see **Chapter 5.1**)



Print current project (see **Chapter 5.2**)



Run detection for representative images only (see **Chapter 16**)



Run detection for all images (see **Chapter 16**)



Select Samples to be displayed (see **Chapter 9.1**)

7.4. Parameters of algorithm

Parameters

DAPI

Nuclei Size: 24

Discrimination by Area: 1

Discrimination by Gray: 0

☐ Autodetect Background Threshold

Threshold Level: 30

Rhodamine

☒ Ring Mask

Interior Radius: 1

Exterior Radius: 3

☒ Identified Cell Mask

Max Growing: 30

Offset from Nuclei: 0

☒ Autodetect Background Threshold

Minimum Level: 0

Maximum Level: 255

Threshold Level: 25

☐ Include Nuclei Label

Figure 48 - Parameter list

The **Parameters** list displays the parameters available for each group of channels (master channel parameters and non-master channel parameters) and allows you to adjust them. For more details see [Chapter 15.2](#).

7.5. Channel list

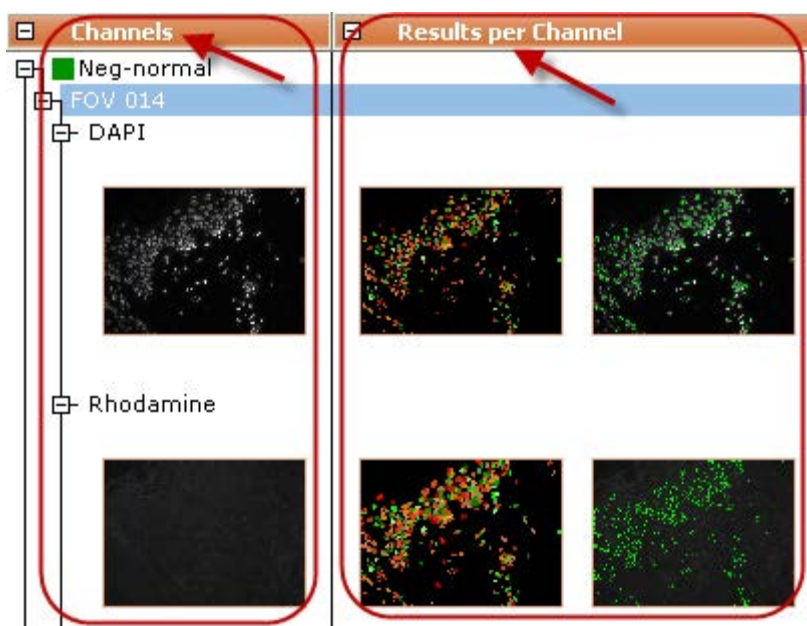
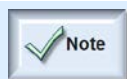


Figure 49 - Channels list

The channel list displays the representative Fields of View. Each **Field of View** has two columns:

- **Channels** - in this column, the original images are displayed.
- **Results per Channel** - in this column, the mask and the overlay images are displayed.



- The mask and overlay images are results of running the detection algorithm.

Running the algorithm

To **run the algorithm** for a specific **sample** or **field of view**, see [Chapter 16](#).

Save to file or copy on the clipboard

To **save to file** or to **copy** to the clipboard an image of a channel, follow the next steps:

- Right click on the image from the channel.
- A context menu will appear.
- Choose **Save as...** or **Copy to Clipboard** from the context menu that appears.

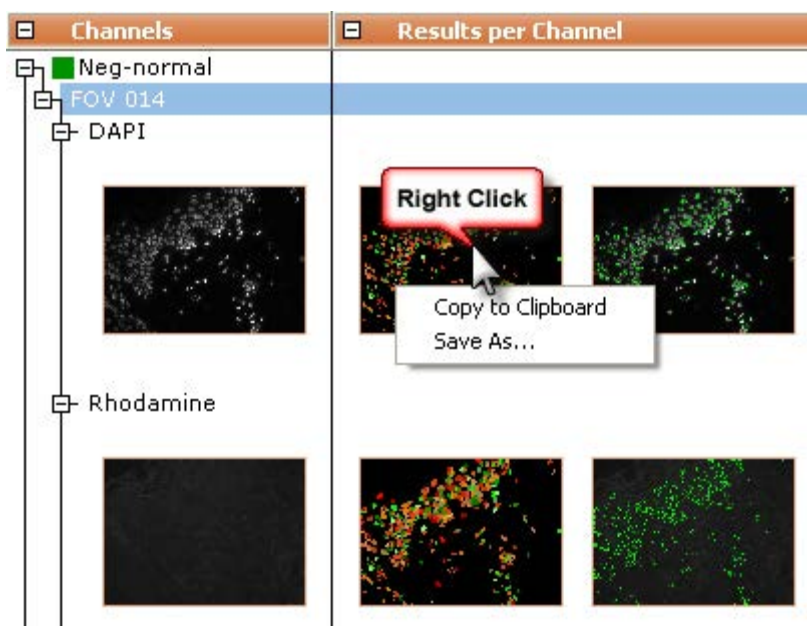


Figure 50 - Channels list: save or copy to clipboard

7.6. Scattergram list

In the **Scattergram list** there are displayed the numerical results of the algorithm, as scattergrams having on their axes output parameters of the algorithms (corresponding to channels). For more details please see **Chapter 10**.

8. Detail Window

The **Detail Window** offers a detailed view for:

- A single field of view

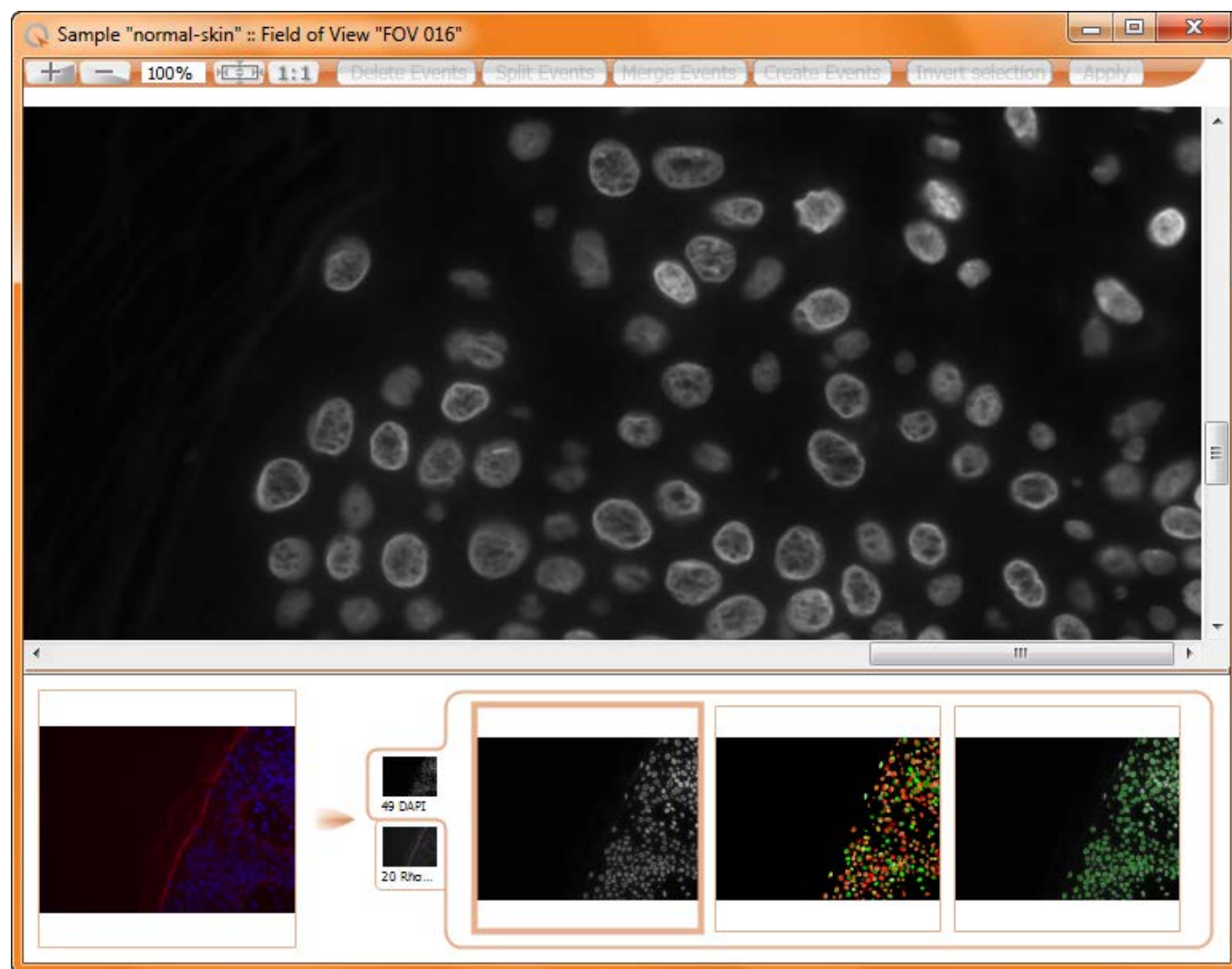


Figure 51 - Detail Window: single field of view

- Multiple fields of view, when backward connection is applied

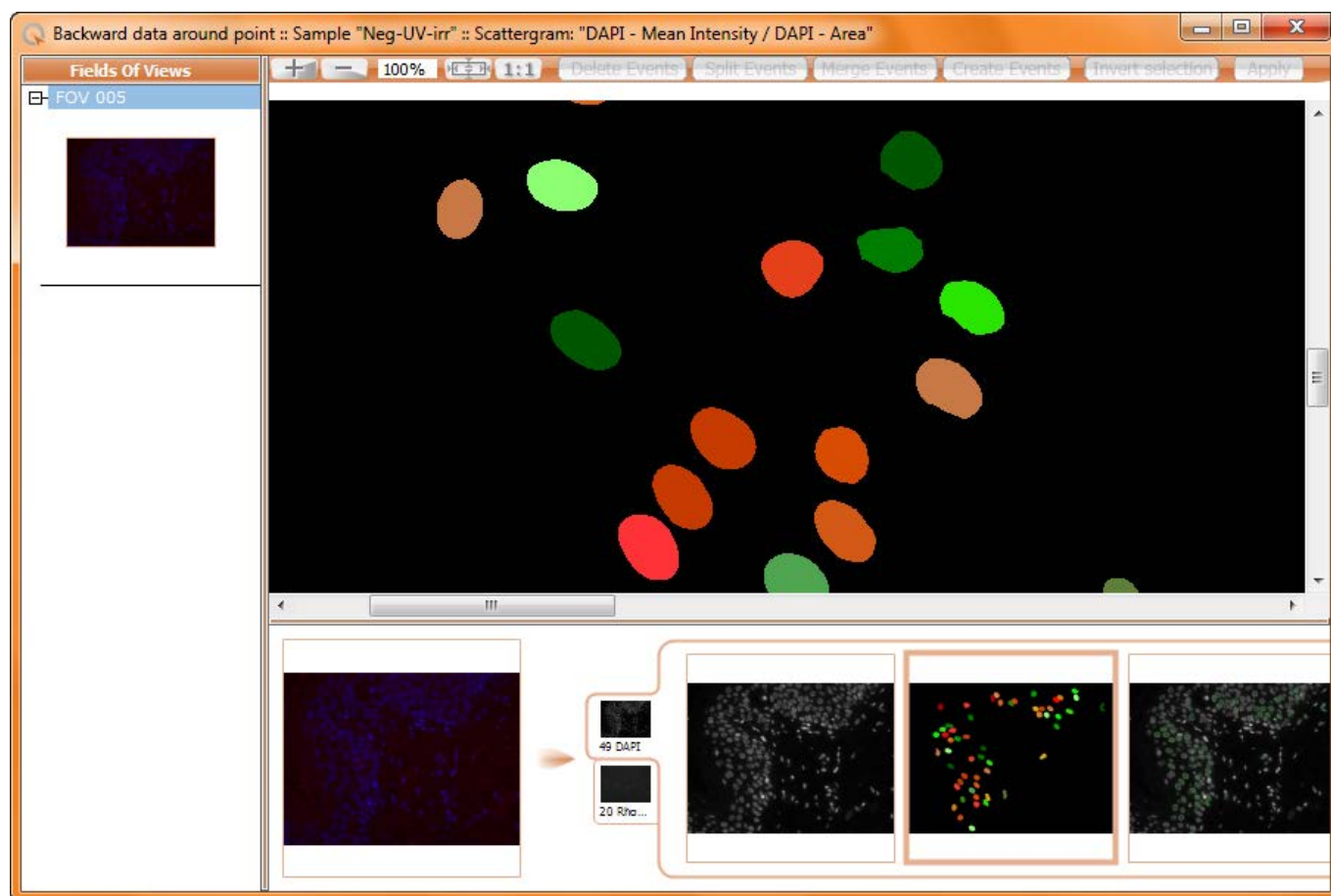


Figure 52 - Detail Window: multiple fields of view (backward connection)

- A scattergram

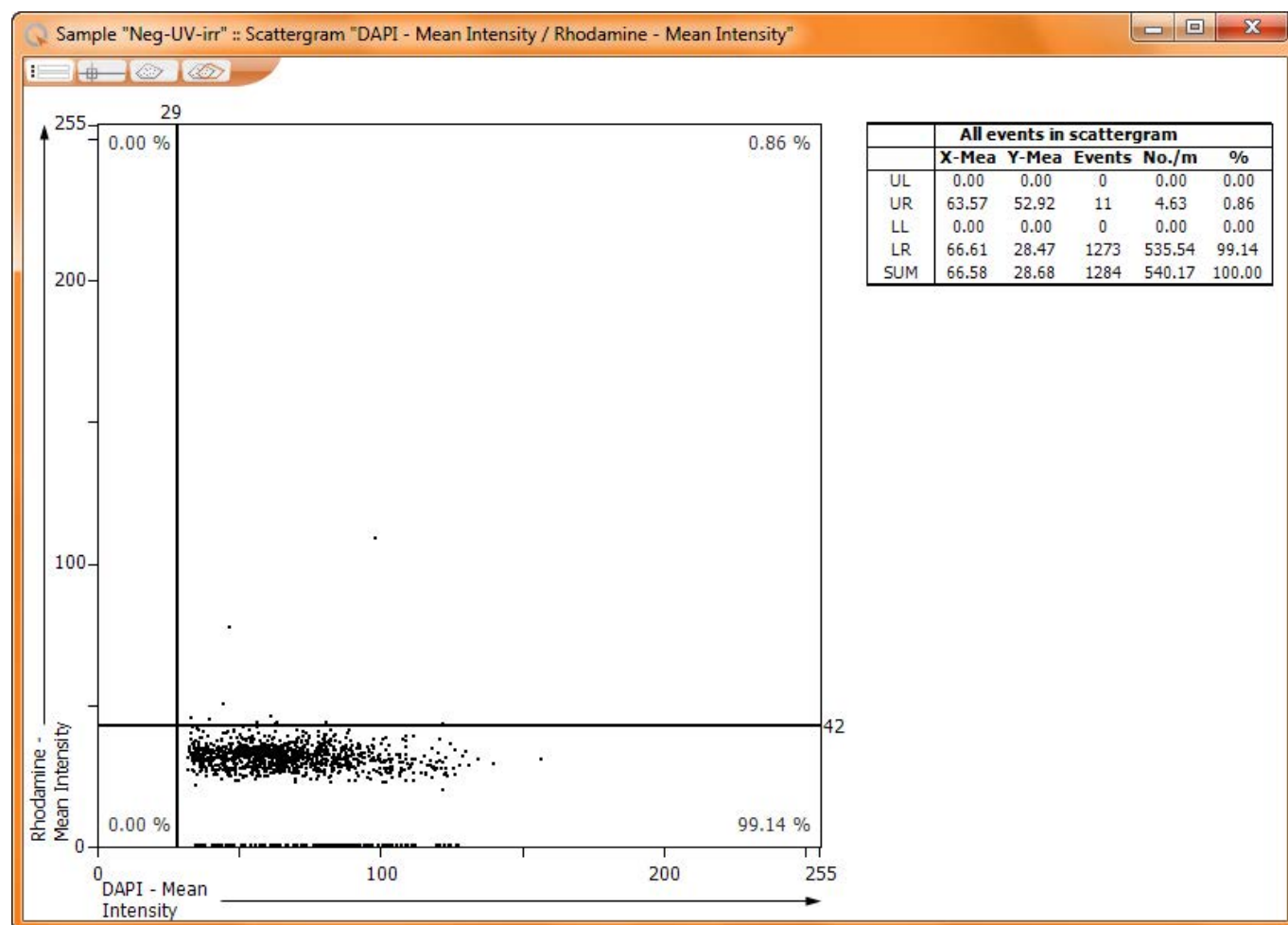


Figure 53 - Detail Window: scattergram

8.1. Detail Window: FOV Viewer Toolbar

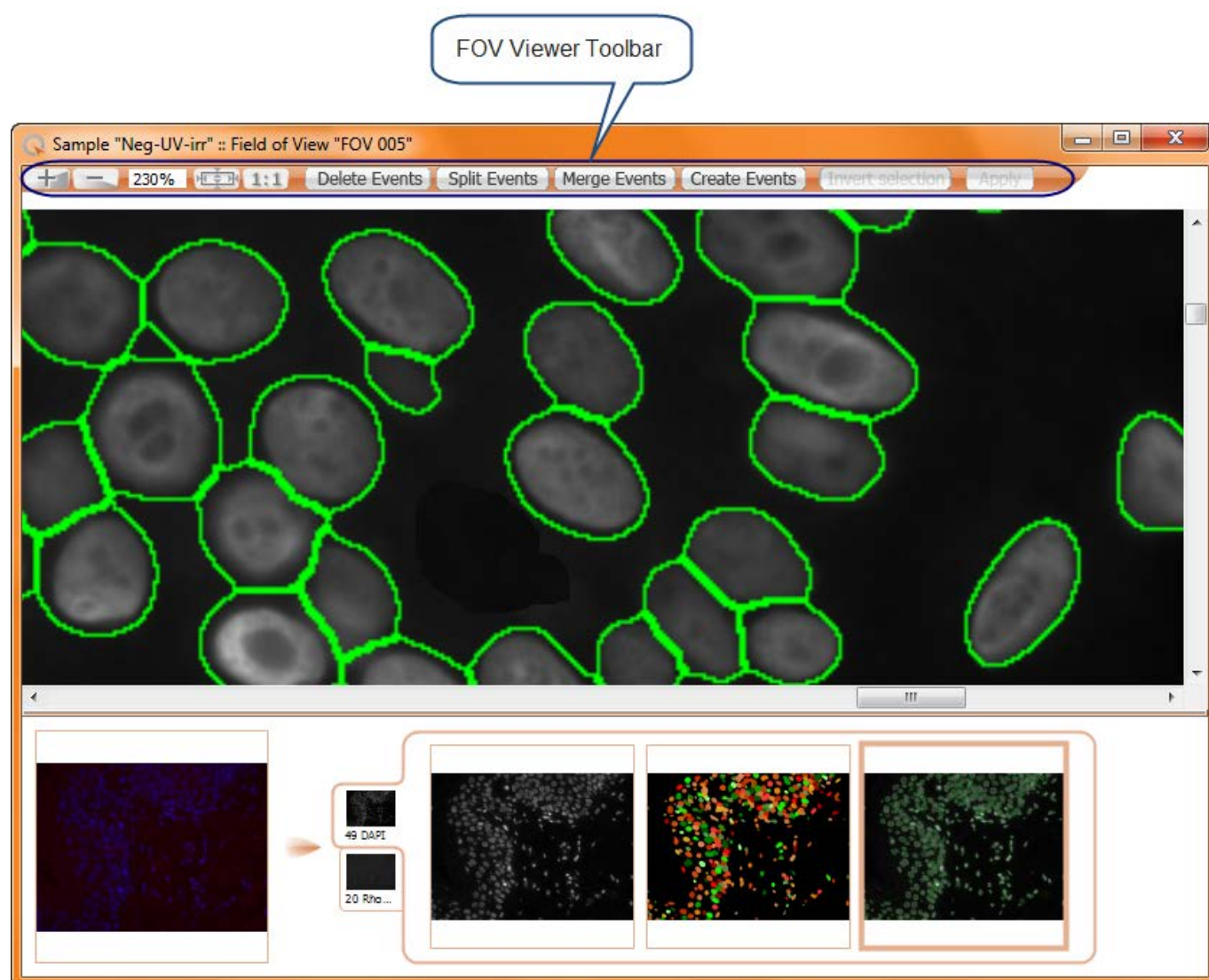











Figure 54 - Detail Window: FOV Viewer Toolbar

-  **Zoom in**
-  **Zoom out**
-  **Fit:** this button scales the image to the size of the window, preserving aspect ratio.
-  **Original size:** this button displays the image at its original size.
-  **Delete Events:** by pressing this button, you enter the **Delete Events** mode.
-  **Split Events:** by pressing this button, you enter the **Split Events** mode.
-  **Merge Events:** by pressing this button, you enter the **Merge Events** mode.
-  **Create Events:** by pressing this button, you enter the **Create Events** mode.
-  **Invert selection:** by pressing this button, you can invert an existing selection. This option is available in **Delete Events** mode, when some events are already selected.

Apply **Apply:** by pressing this button, you can effectively apply the following operations: delete, split, merge and create events.

8.2. Delete events

To delete events from your image follow the steps below:

1. Select the overlay image of the master channel (simply click on it).
2. Press **Delete Events** button.
3. Select the events you want to delete.

To select multiple events to be deleted:

- Holding **Control** key pressed, click on the events by using the left mouse button.
 - Holding left mouse button pressed, draw a region that includes the events (only the events that are totally inside the region will be selected and deleted).
4. Use **Invert Selection** button if you want to invert your selection.
 5. Press **Apply** button to delete selected events.

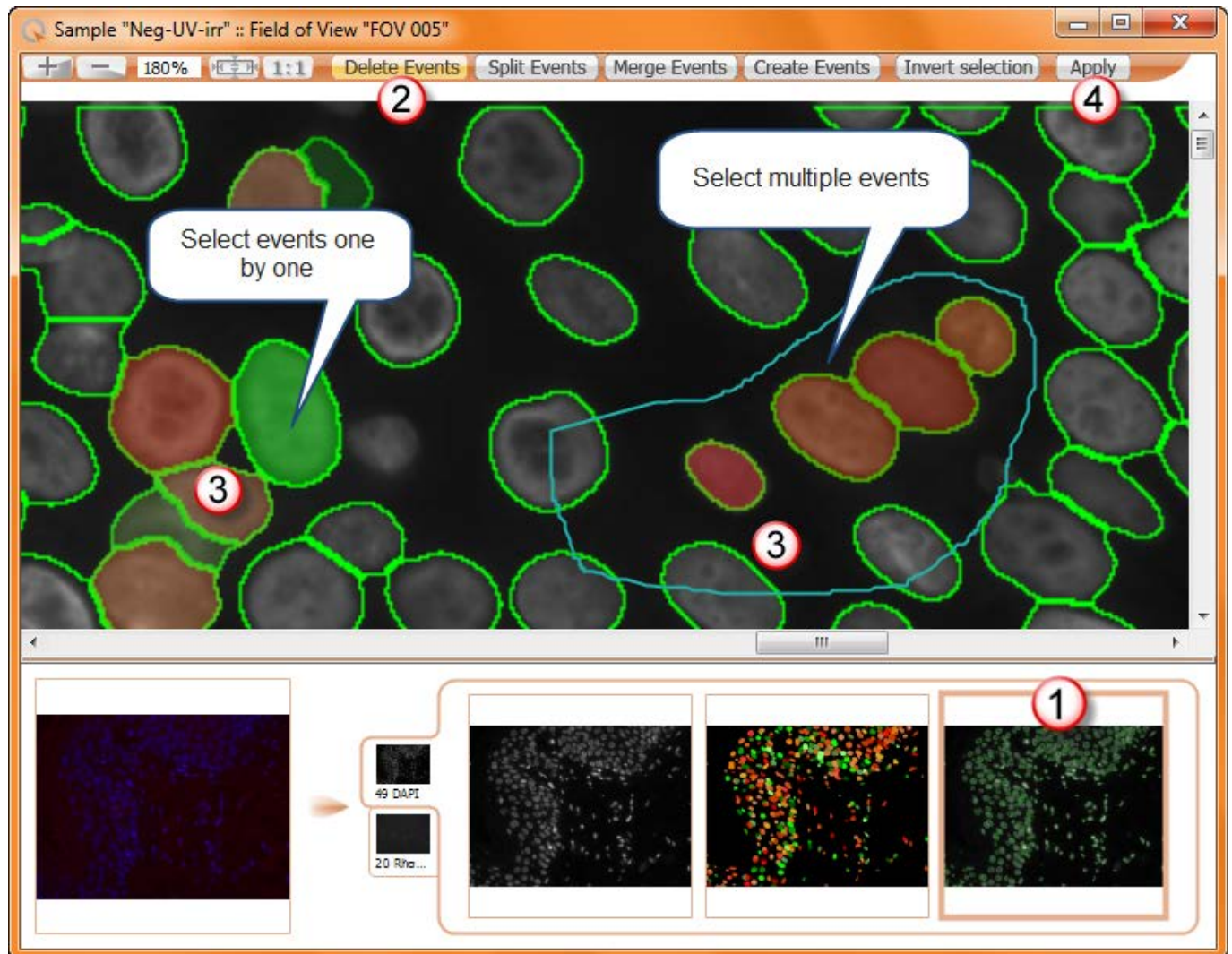
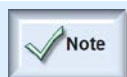


Figure 55 - Detail Window: delete events

8.3. Split events

To split events, follow the steps below:

1. Select the overlay image of the master channel (simply click on it).
2. Press **Split Events** button.
3. Holding the left mouse button pressed, draw lines across the events you want to split.
4. Press **Apply** button to split the events.



- You cannot split events on images obtained as a result of backward data computation.

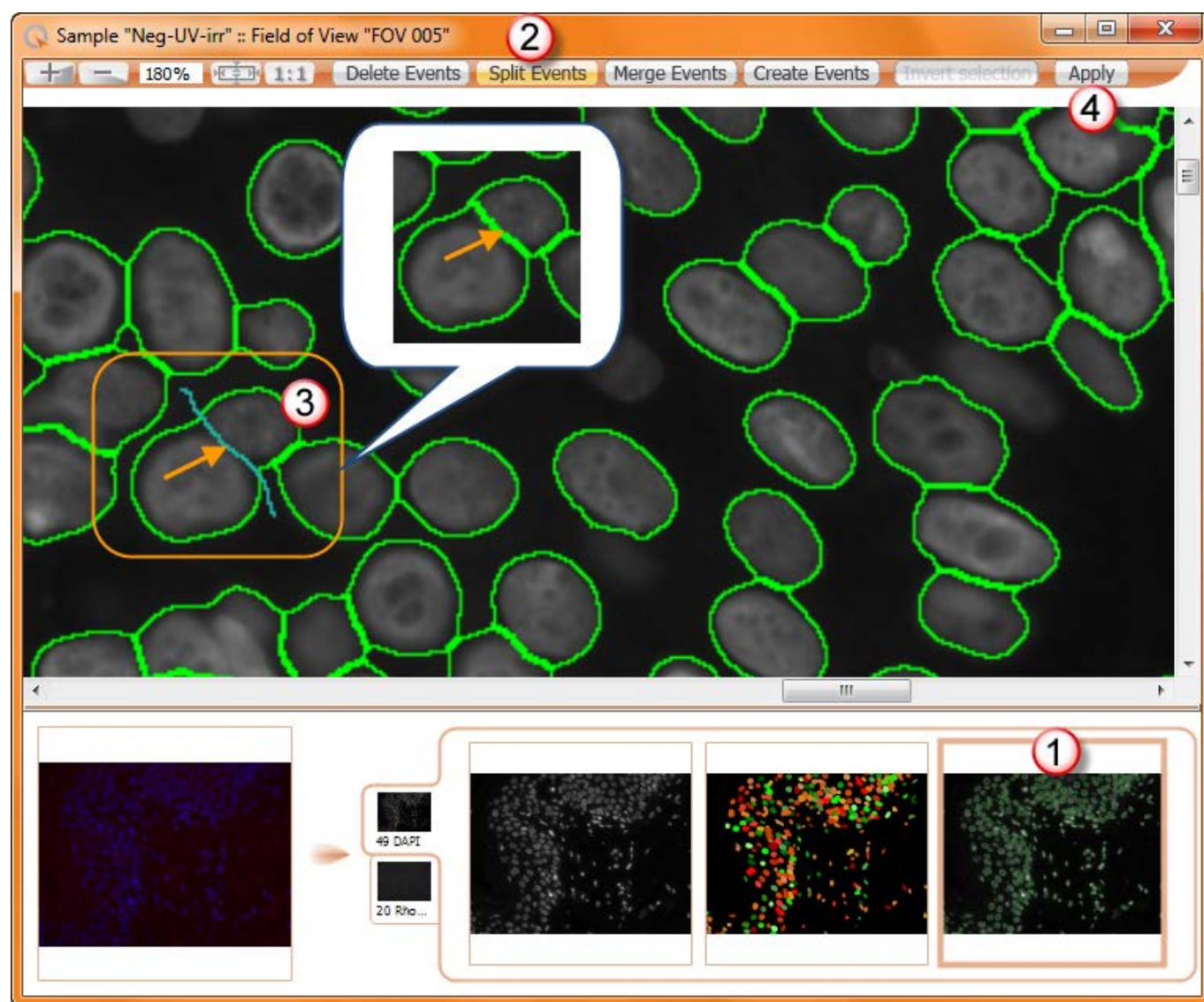


Figure 56 - Detail Window: split events

8.4. Merge events

To merge events, follow the steps below:

1. Select the overlay image of the master channel (simply click on it).
2. Press **Merge Events** button.
3. Create groups of events to be merged:
 - Click on each event using left mouse button to include them in the group. Only neighboring (touching) events can be selected in the same group for merge.
 - Click using right mouse button in order to close the group.
4. Press **Apply** button to merge the events.

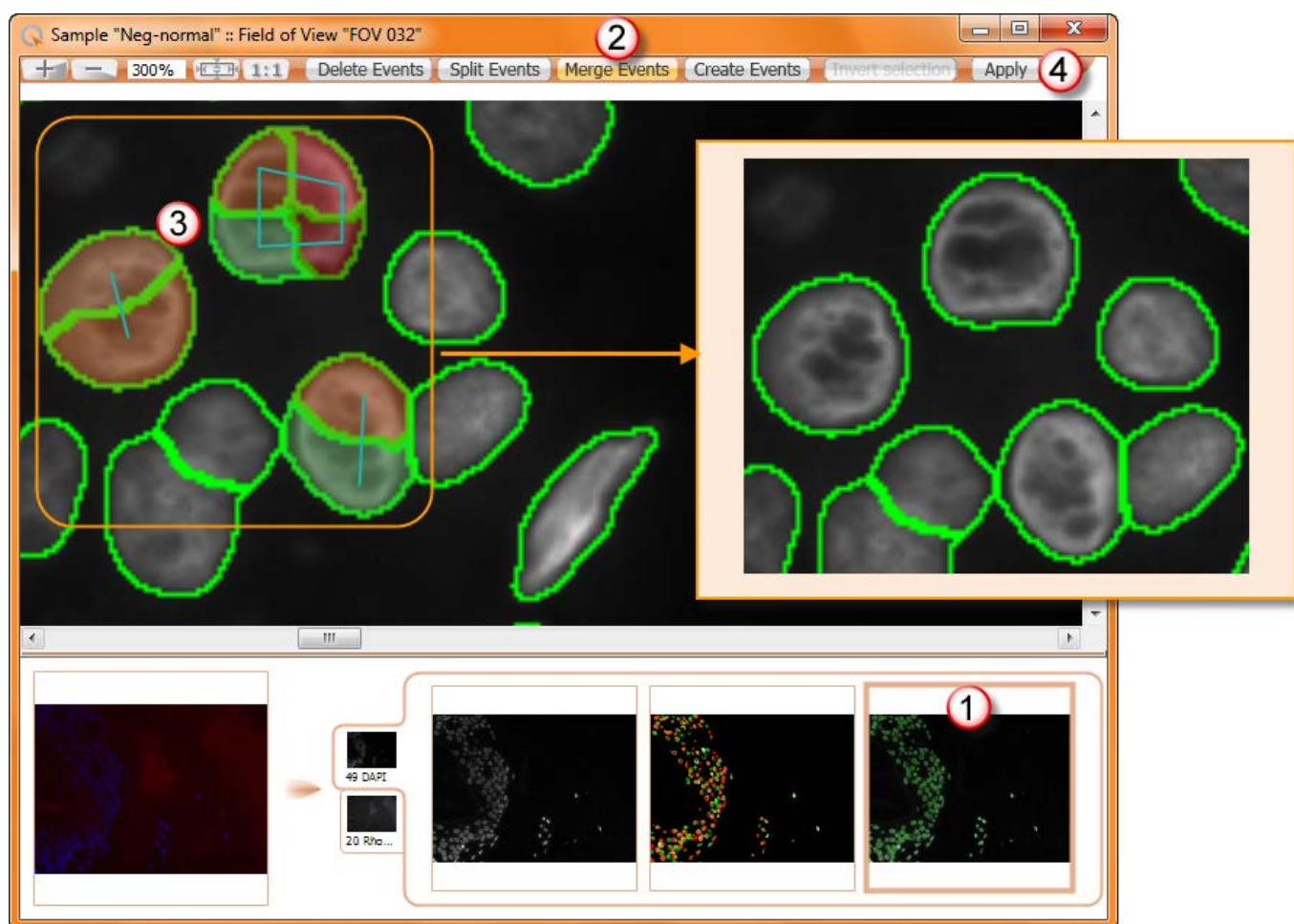


Figure 57 - Detail Window: merge events

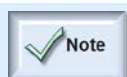
8.5. Create events

To create events, follow the next steps:

1. Select the overlay image of the master channel (simply click on it).
2. Press **Create events**.
3. Holding the left mouse button pressed, draw each individual event that will be created.
4. Press **Apply** button to create the events.



Figure 58 - Detail Window: delete events



- You cannot create events on images obtained as a result of backward data computation.

8.6. Contextual menu

When an image of the channel is displayed, you can right click on any event to see the contextual menu. This contextual menu has two options:

- Delete event
- View event data

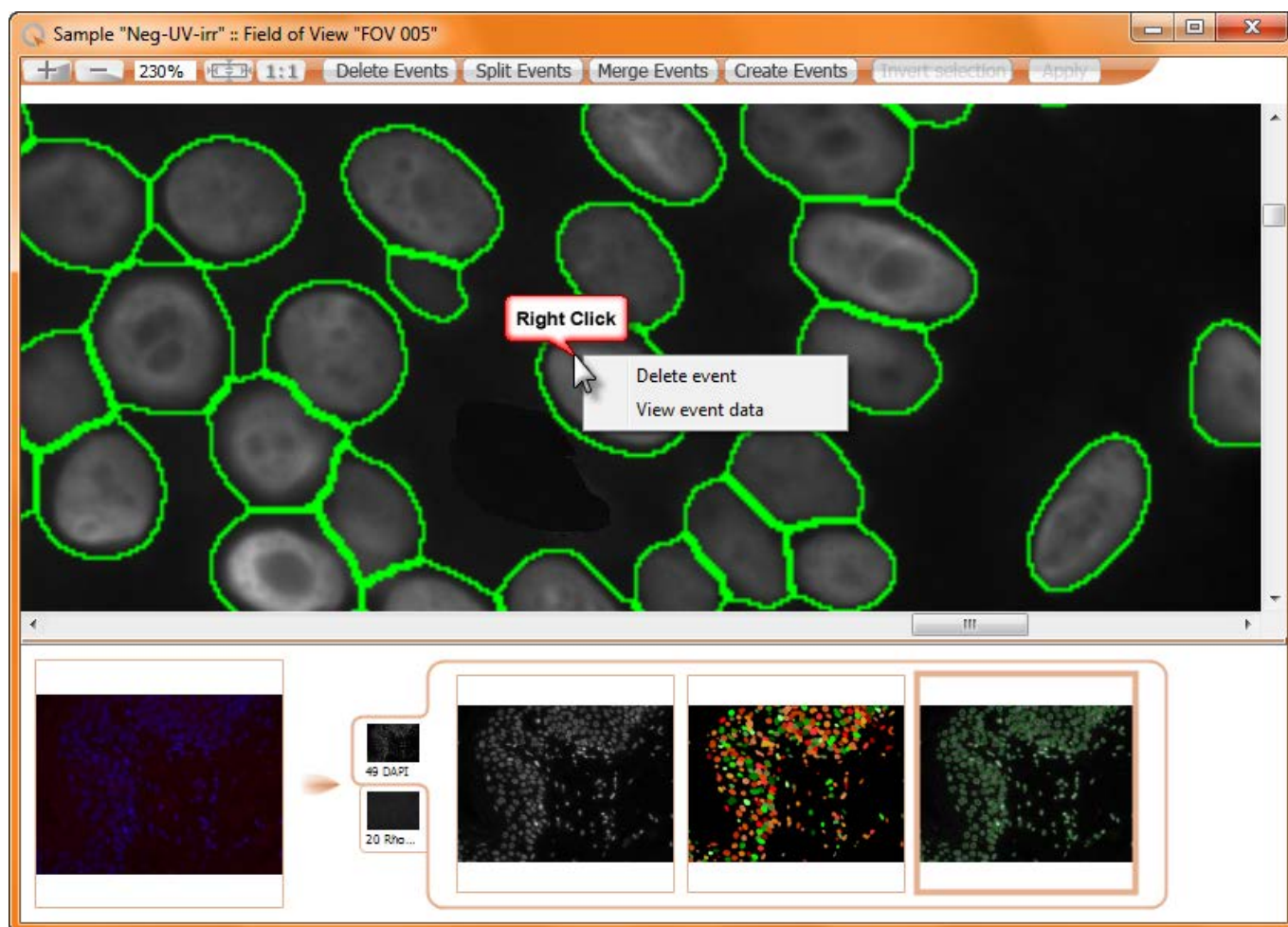


Figure 59 - Detail Window: contextual menu

- You can delete that event by choosing **Delete event**.
- You can view the numerical results (raw data) for that event if you choose **View event data** (see also [Chapter 17.2](#)).

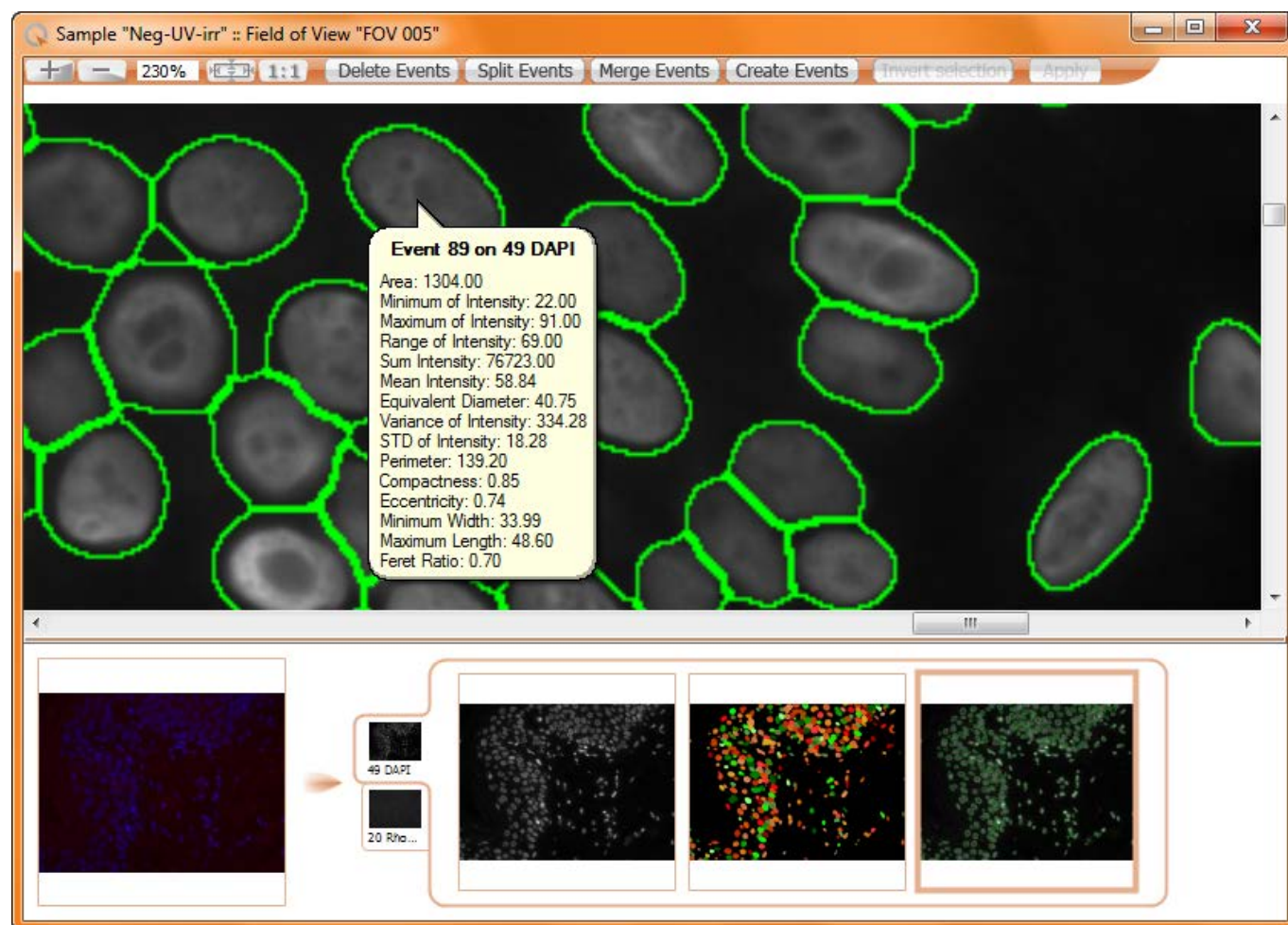


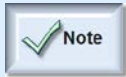
Figure 60 - Detail Window: View event data

While visualizing event data, forward connection is activated. For more details, see [Chapter 11.1](#).

9. Samples

9.1. Select Sample to be Displayed

Press **Select Samples** button from the main **Toolbar** to access a dropdown list where you can select the samples you want to have displayed in the Main window.



- **TissueQuest** allows you to have simultaneously displayed a maximum number of 10 samples.

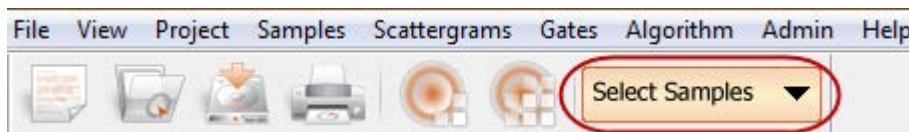


Figure 61 - Select Samples button

In the dropdown list, simply mark the checkbox corresponding to the sample(s) you want to display.

To display the previous or next block of samples, use “<<” and “>>” buttons.

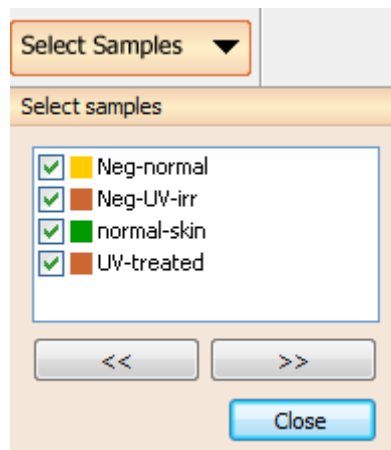


Figure 62 - Select Samples: dropdown list

9.2. Sample linking

Linking samples means grouping two or more samples so they will share the same properties:

- They have the same scattergrams: a scattergram added to a sample is automatically propagated to all the samples belonging to the link of samples.
- The scattergrams have the same properties:
 - The same cutoff values: the cutoff values set for a scattergram of a sample are automatically propagated to the same scattergram in all the samples belonging to the link of samples;
 - The same defined gates: the gate inserted in a scattergram of a sample is automatically propagated to the same scattergram in all the samples belonging to the link of samples;
 - The same input gates.

Sample linking can link only compatible samples. A set of channels are compatible if:

- They have the same number of channels.
- Their channels have associated the same markers or markers belonging to the same marker family.

For **example**, let's consider the following:

- *Sample1* contains two channels (CH_a and CH_b) with markers DAPI and Rhodamine, respectively.
- *Sample2* contains two channels (CH_c and CH_d) with markers Rhodamine and DAPI, respectively.
- In this case, the two samples are compatible because their channels have associated the same markers: CH_a and $CH_d \rightarrow$ DAPI, CH_b and $CH_c \rightarrow$ Rhodamine. As you may notice, the order of the channels is not important.

Access **Samples** → **Linking...** from the main **Menu** toolbar in order to use the **Linking** option.

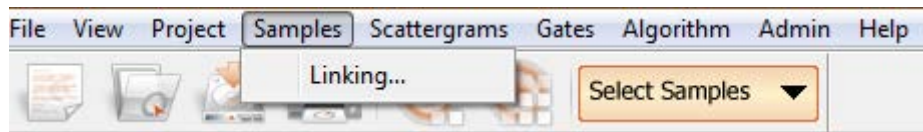


Figure 63 - Samples: Linking option

The **Sample Linking** dialog will appear.

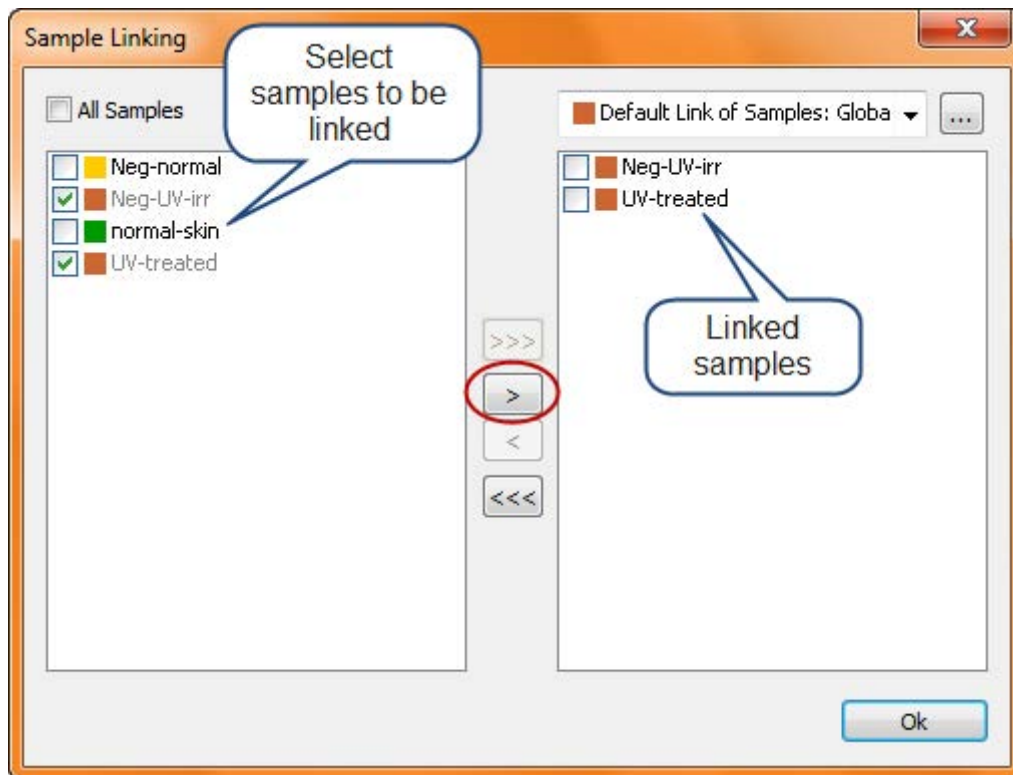



Figure 64 - Sample linking dialog

- In the left column, you will find the list of available samples.
- Mark the checkbox for the samples you want to link, then press  button to move the respective samples into the right column in order to link them.



- If you cannot mark the checkbox for a sample, it means the sample is not compatible with the previously selected samples.

- Use the button to move all samples to the right column.
- Use the button to send a sample back to the left column or the button to send all samples to the left column.



- As you may notice, each link of sample has associated a color. This color is displayed in front of the names of link and samples. This helps you easily identify the samples belonging to each link of the sample.

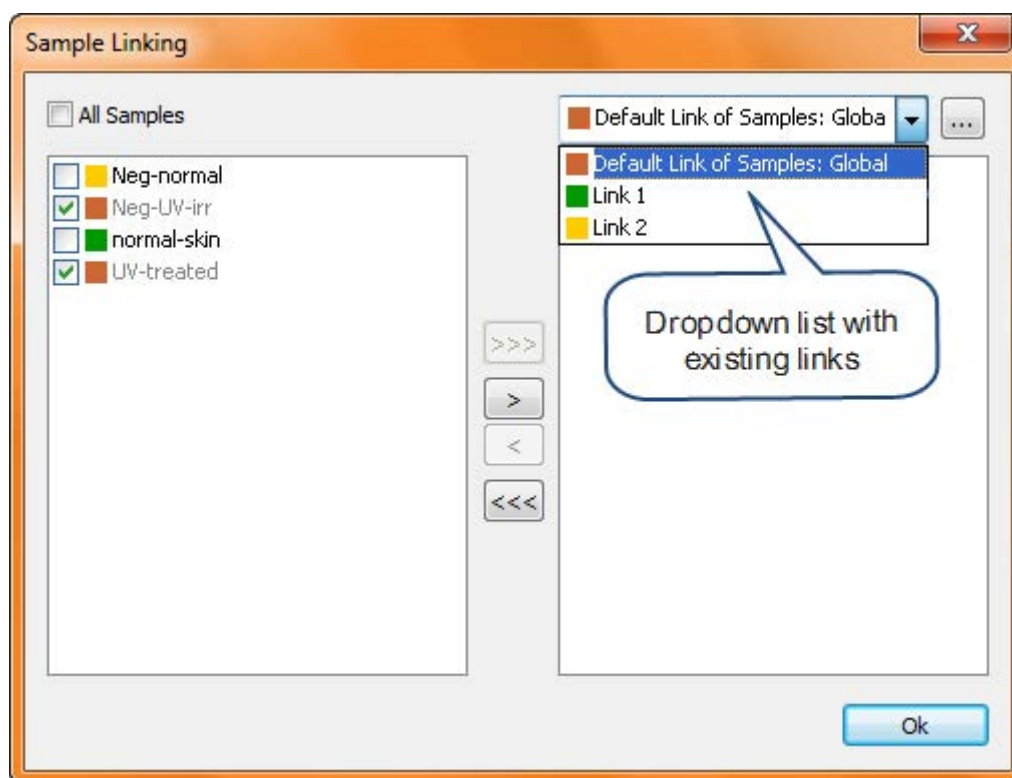



Figure 65 - Sample linking dialog: dropdown list

Manage links

Press the  button to access **Sample Links** dialog.

Here, you can visualize details about the existing links, modify them or create new links.

- To **modify** existing links, select the desired link in the **Link Name** section, then edit the corresponding field(s): Name, Description, Color, then press **Update** button.
- To create a **new link**, fill the Name and Color fields (mandatory) and Description (optional), then press **Add** button.
- To remove a link, click on the desired link in the **Link Name** section, then press **Remove** button.

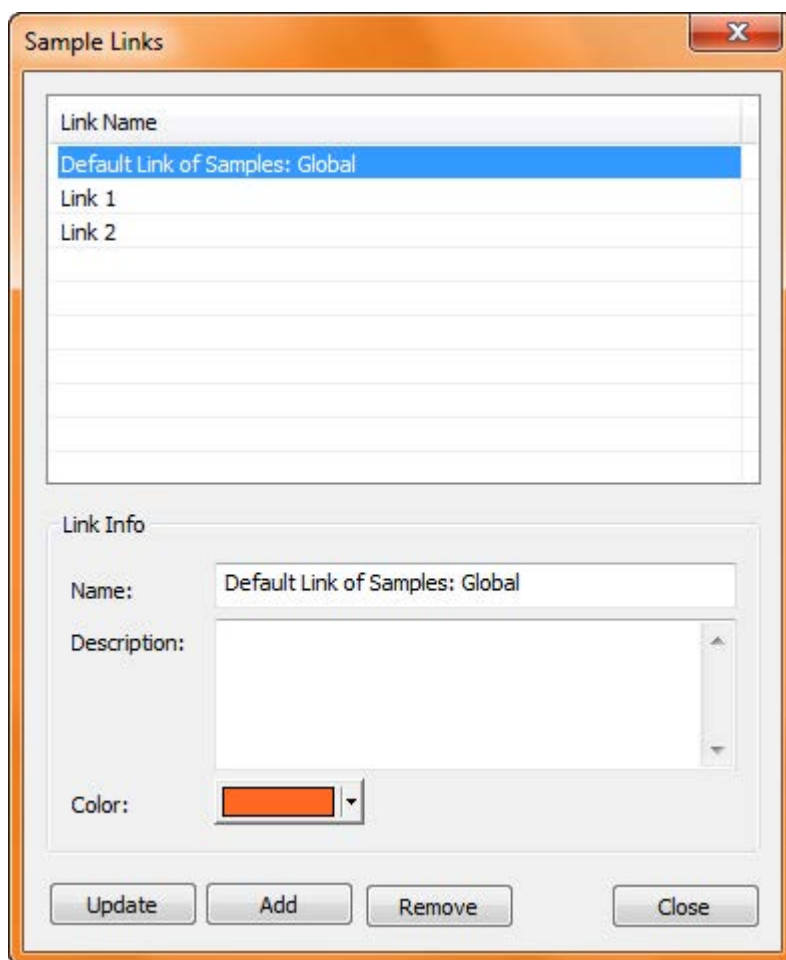


Figure 66 - Sample links dialog

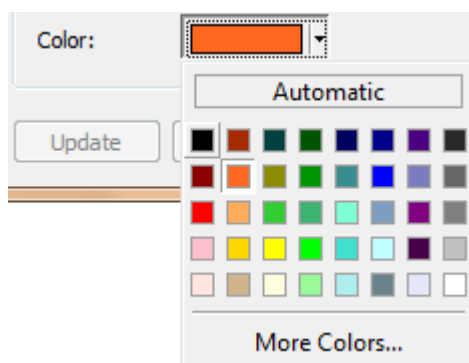


Figure 67 - Sample links dialog: edit color

10. Scattergrams

10.1. Manage scattergrams

To manage scattergrams, go to **Scattergrams** option in the **Menu** bar, then choose **Manage...**

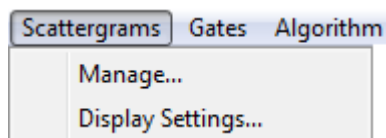


Figure 68 - Manage scattergrams menu

The following dialog will appear:

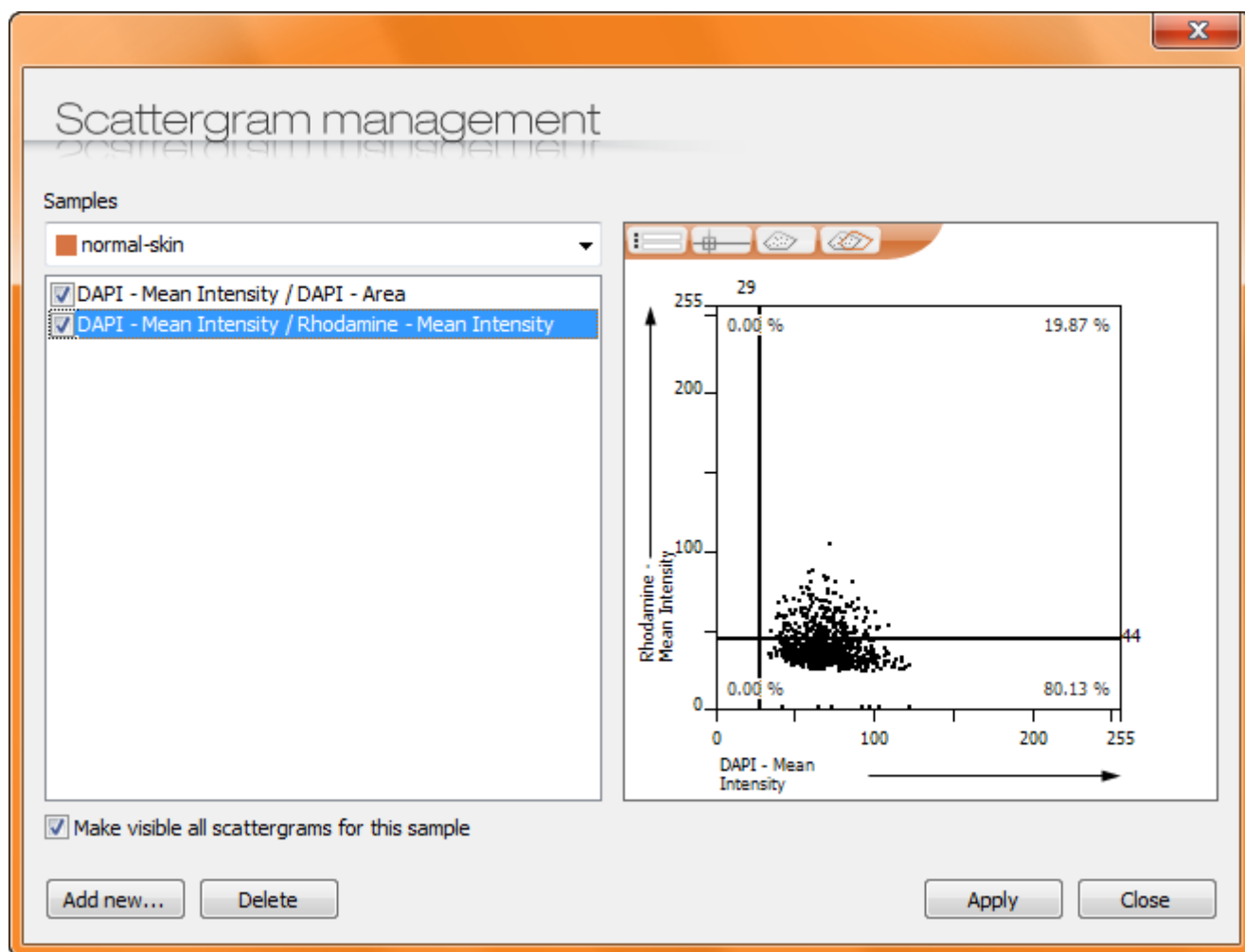


Figure 69 - Scattergram management dialog

10.1.1 Add scattergram

- Press **Add new...** button from **Scattergram management** dialog.

- Set the properties of the new scattergram in the **Scattergram properties** dialog box.
- Press **Ok** to create the new scattergram.

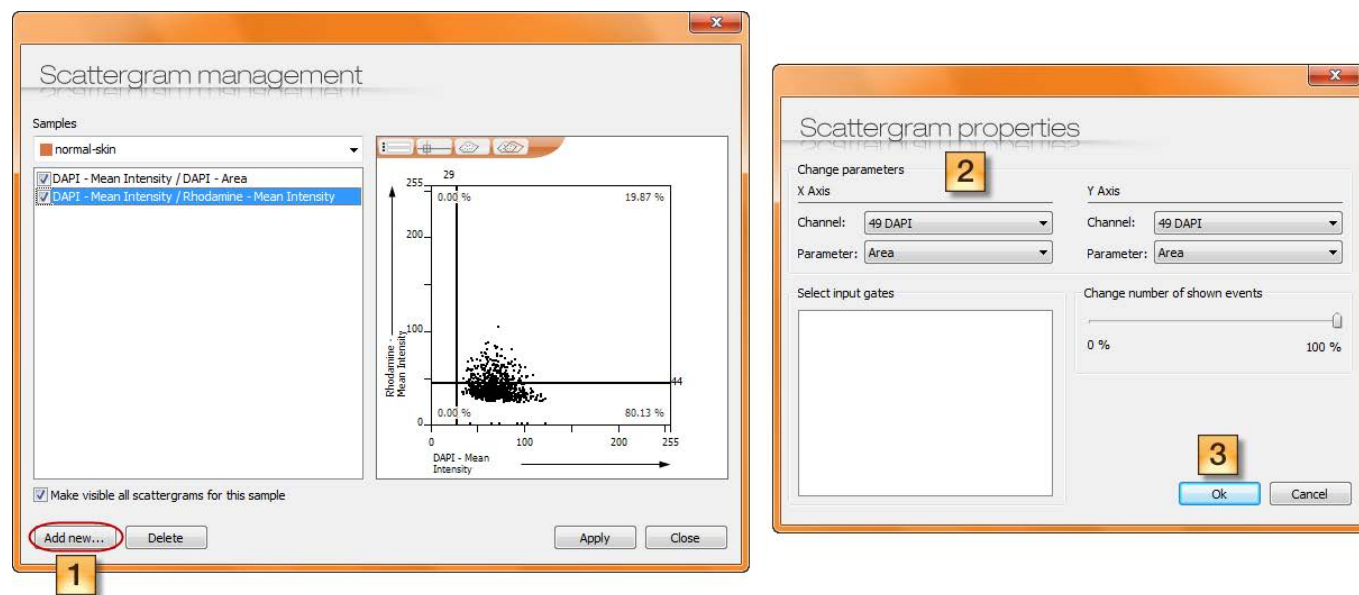


Figure 70 - Manage scattergram: create scattergram

10.1.2 Delete Scattergram

- Select the scattergram you want to delete in **Scattergram management** dialog. On the right of the dialog box, you can see the image of the selected scattergram.
- Press **Delete** to delete the scattergram.

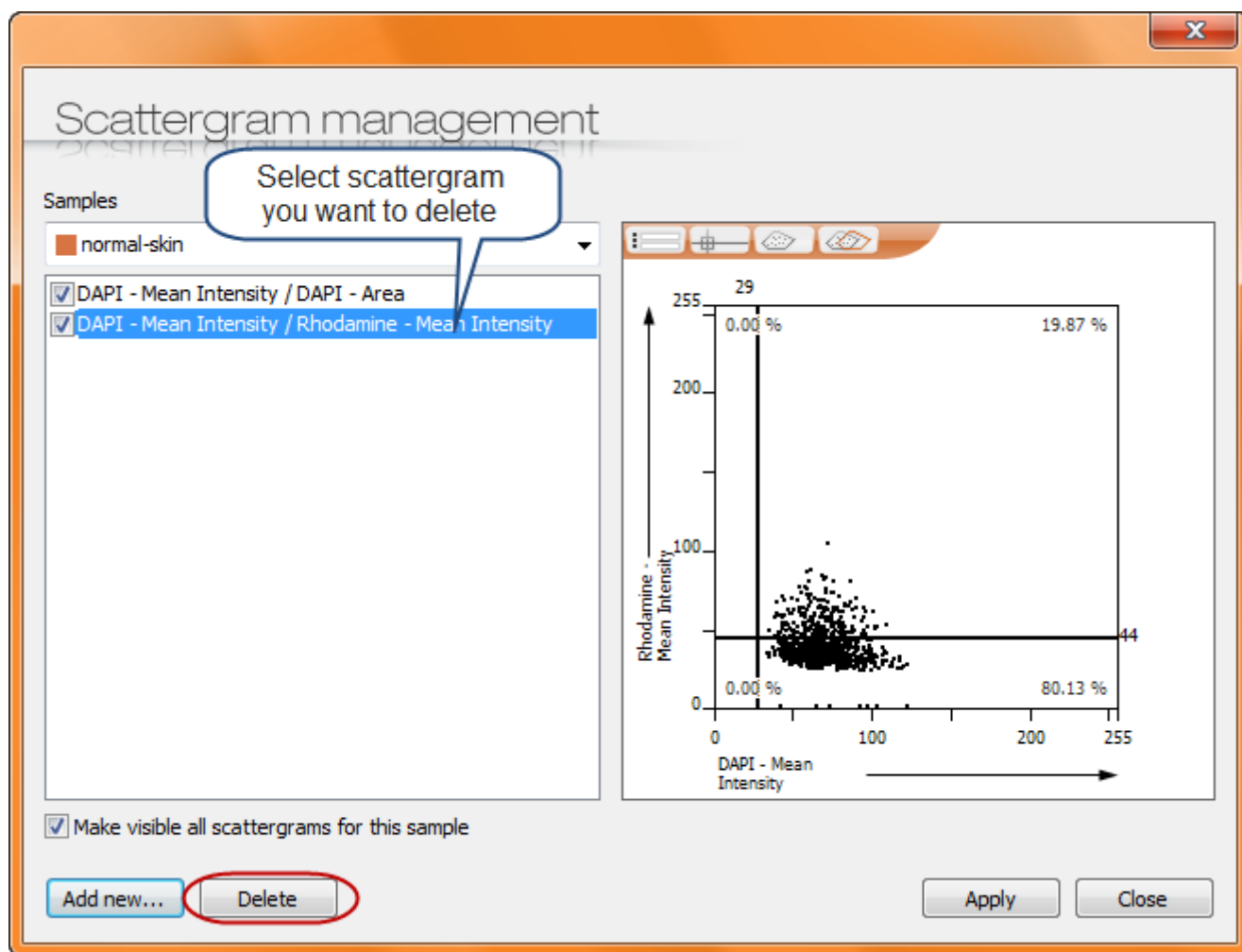


Figure 71 - Manage scattergram: delete scattergram

10.1.3 Show/Hide scattergram

You have two options to **hide** a scattergram.

1. From **Scattergram management**, uncheck the corresponding checkbox for the scattergram you want to hide, then press **Apply**.

- If you want to display all the scattergrams from the sample, check **Make visible all scattergrams for this sample**. Press **Apply**. The hidden scattergram(s) will be displayed again.

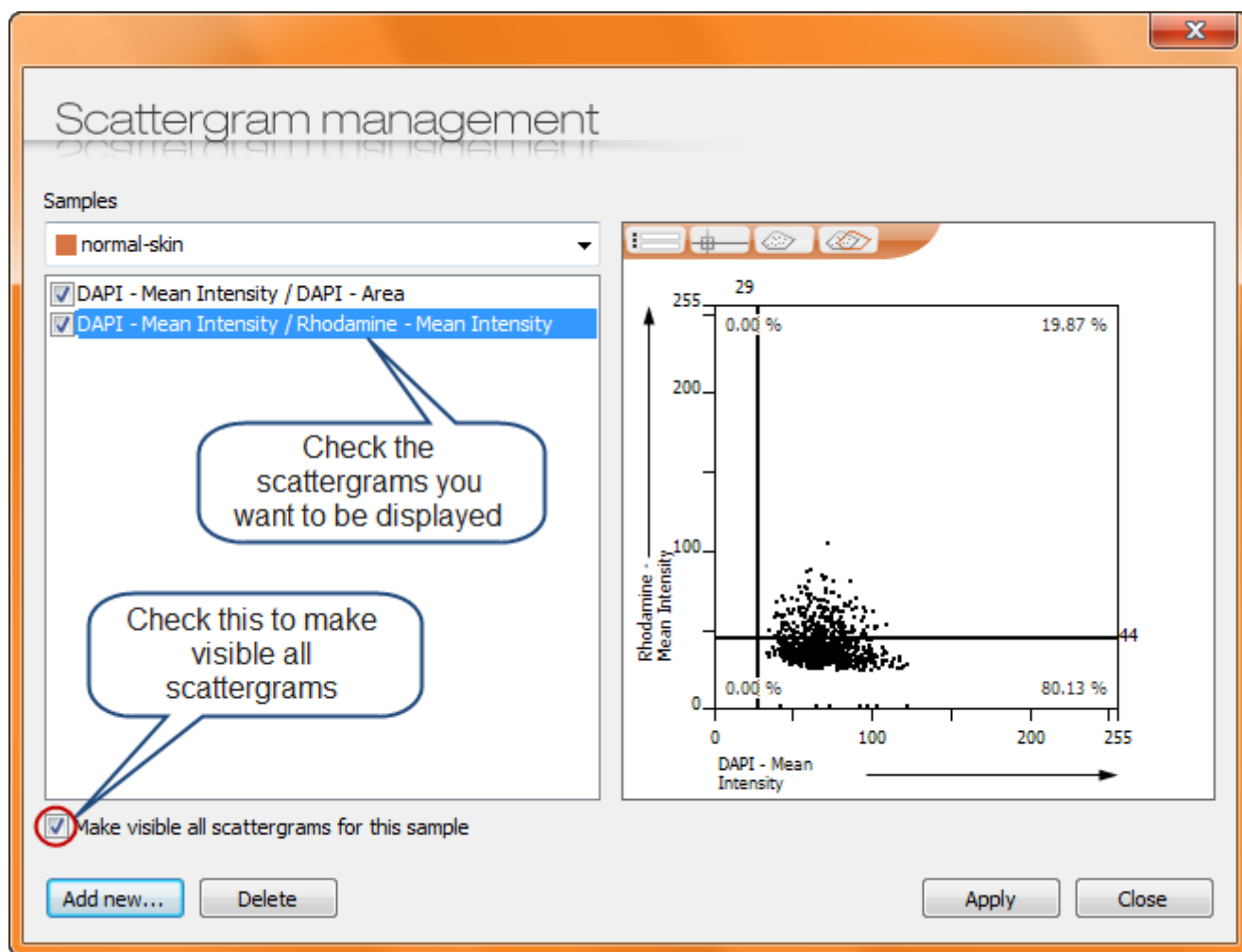



Figure 72 - Manage scattergram: show hidden scattergram, step 2

2. Use **Hide scattergram** button () in the scattergram toolbar.

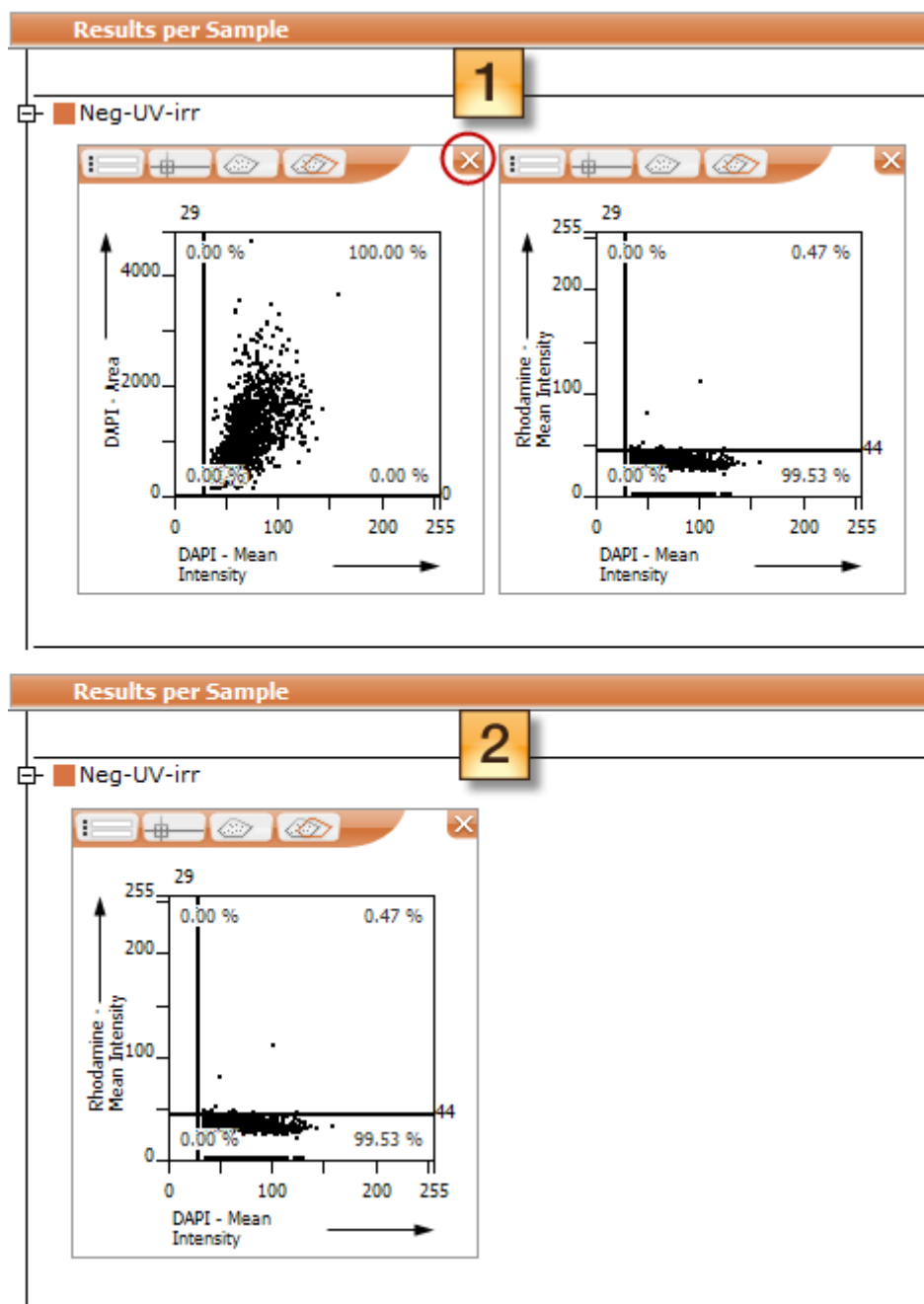


Figure 73 - Manage scattergram: hide scattergram

10.2. Display settings

To change the scattergram display settings select **Scattergrams** from the application's main menu.

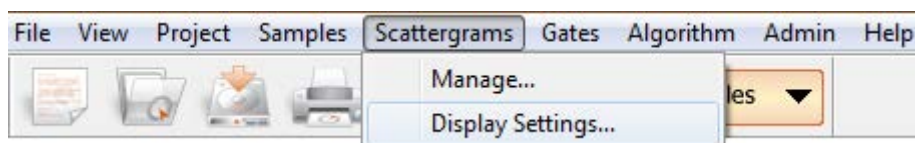


Figure 74 - Scattergrams menu

From the menu that appears, choose **Display settings...** and a dialog will appear.



Figure 75 - Scattergrams: Display settings dialog

Here, you can set the **dot size** and also select one of the three available **dot styles**:

- rectangle
- circle
- cross

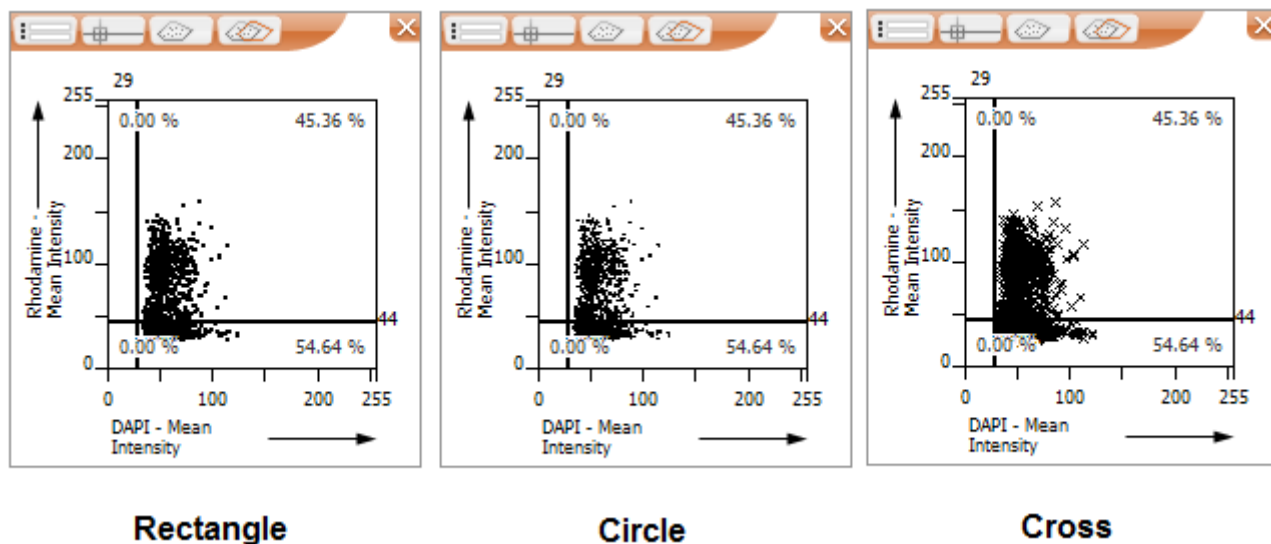


Figure 76 - Scattergrams: dot styles examples



- When changing the dot styles, the size of the events in the scattergrams can be altered.

10.3. Scattergram Toolbar

In each scattergram, the **scattergram toolbar** is placed above the chart area.

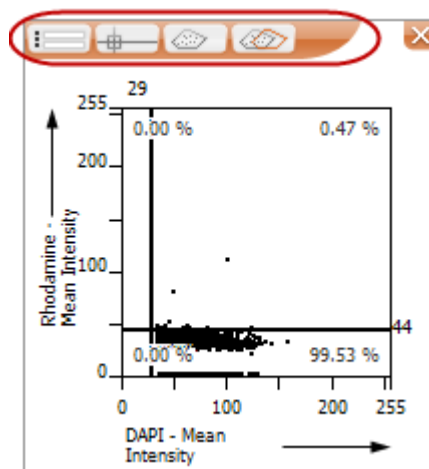



Figure 77 - Scattergrams: toolbar

Actions in a scattergram

For each scattergram you can perform the following **operations**:

1. **Change parameters:** by pressing , **Scattergram properties** dialog will appear. In this dialog you can change the parameters for the x-/y-axis, select the input gates and change the number of shown events.

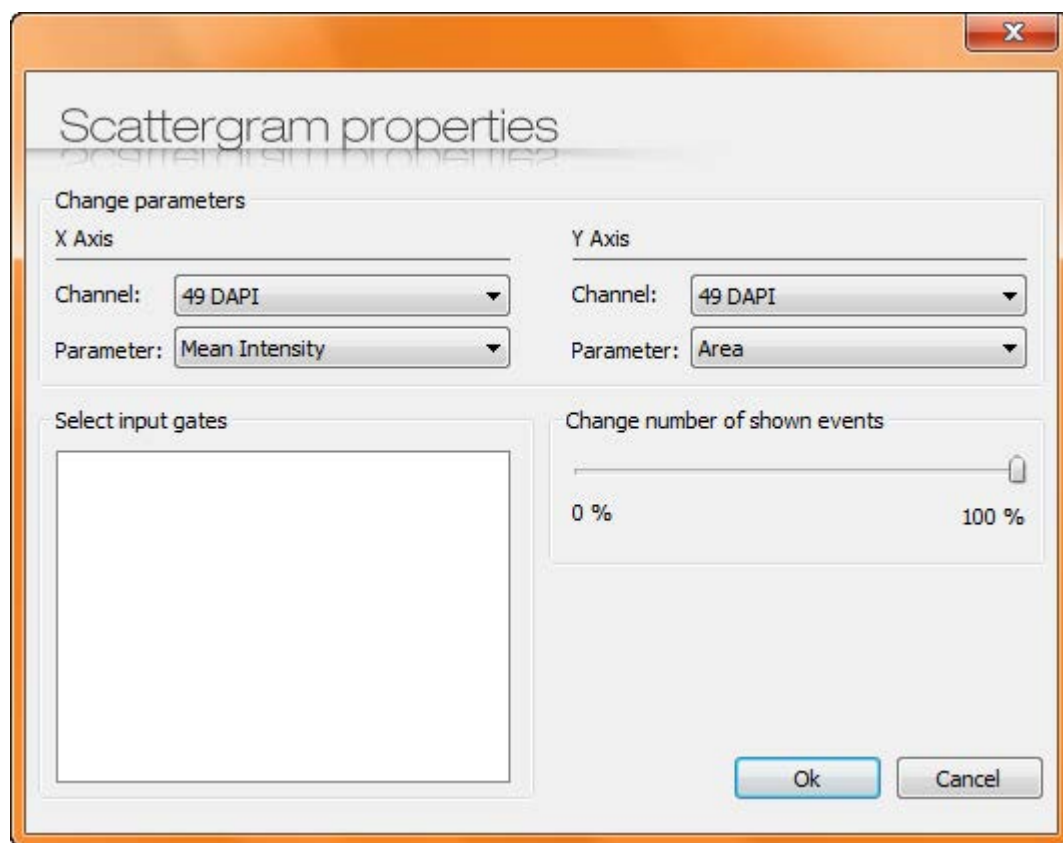



Figure 78 - Scattergrams: Scattergram properties dialog

- To **change the parameters** for the x- and y- axis, select from the dropdown list corresponding to the axis the channel and the measurement. For more details see [Chapter 17.2.1](#) and [Chapter 17.2.2](#).
- Optionally, you can set the input data for the scattergram by selecting **input gates**. If no input gate is selected, all events are taken and shown inside the plot.
- Optionally, you can set the **number of shown events** (percent). This tool is useful when the number of events is very high and the dot-clouds become too dense to be interpreted. For example, by setting the slider to 50 percent, only every second event will be shown.

2. **Set Cutoff** (by pressing ): for details, see [Chapter 10.4.1](#).

3. **Add gate** (by pressing ): for details, see [Chapter 10.5.1](#).

4. **Edit gate** (by pressing ): for details, see [Chapter 10.5.3](#).

10.4. Cutoffs

A **cutoff** is a numerical parameter, used as a threshold value in a scattergram in order to determine positive and negative measure events (e.g. events with staining intensities exceeding or not the staining intensity of a negative control).

10.4.1 Add cutoff

- Click **Set cutoff** button in the scattergram toolbar.

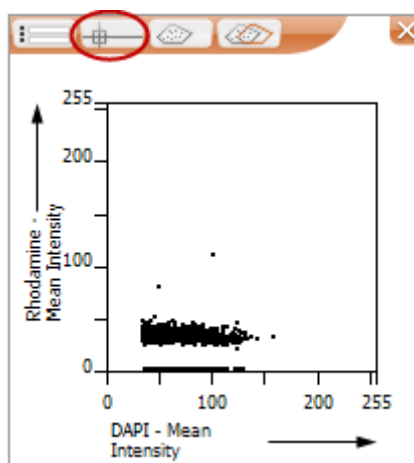


Figure 79 - Add cutoff: step 1

- Move the mouse to the position where you want to have the cutoff. During this operation, the mouse cursor is captured inside the diagram area.

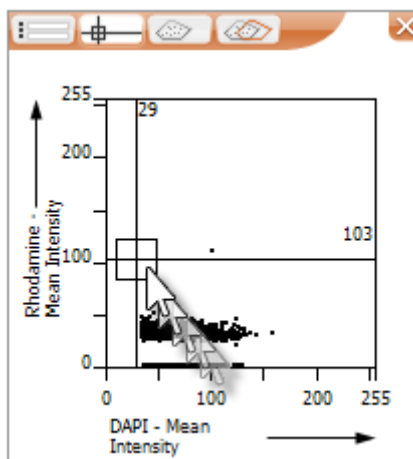


Figure 80 - Add cutoff: step 2

- Finally, the cutoff is determined by clicking the left mouse button on the desired position in the diagram.

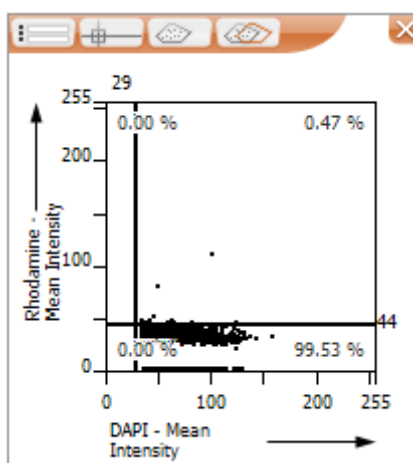


Figure 81 - Add cutoff: step 3

10.4.2 Modify cutoff

- Move the mouse cursor over the cutoff you want to modify. As a result, the cursor changes to a pointed arrow shape (↔↕). It is possible to move: both cutoffs (horizontal and vertical); only the vertical cutoff; only the horizontal cutoff.

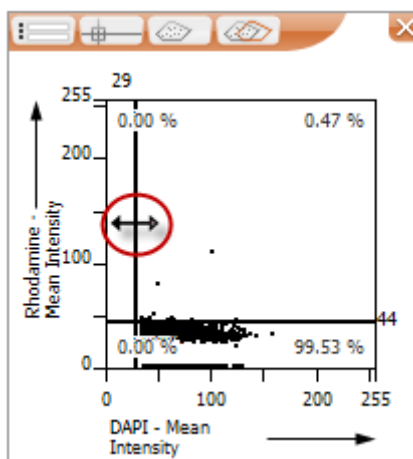


Figure 82 - Move cutoff: step 1

- Press left mouse button on the cutoff.
- Holding left mouse button pressed, move the cutoff to the desired new position.

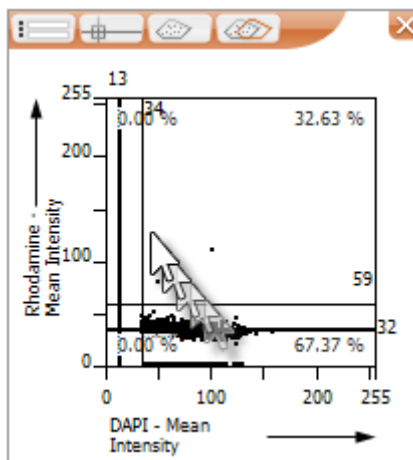


Figure 83 - Move cutoff: step 2

- Finish the operation by releasing left mouse button.

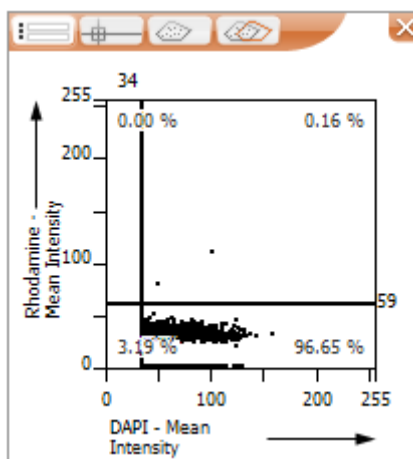



Figure 84 - Move cutoff: step 3

10.4.3 Delete cutoff

- Move the mouse cursor over the cutoff that you want to delete. As a result, the cursor changes to a pointed arrow shape ().

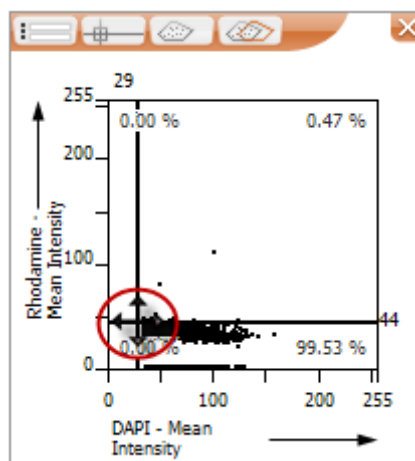


Figure 85 - Delete cutoff: step 1

- Click on the cutoff using the right mouse button to see the contextual menu. Select **Delete cutoff** entry in the menu. The cutoff in the scattergram will immediately disappear.

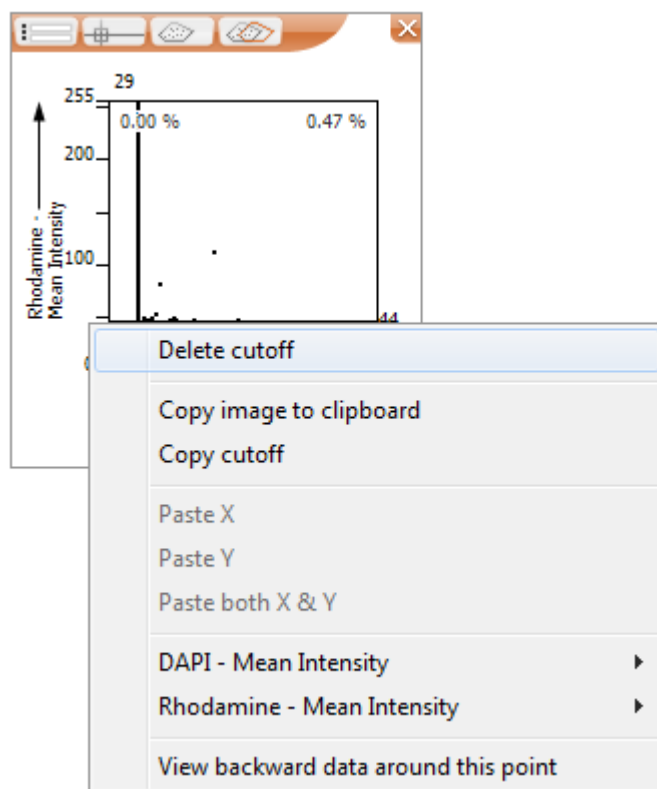
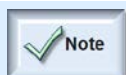
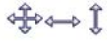


Figure 86 - Delete cutoff: step 2



- The cutoff will be deleted from all the scattergram in which it appears.

10.4.4 Copy cutoff

- Move the mouse over the cutoff that you want to copy. As a result the cursor changes to a pointed arrow shape ().

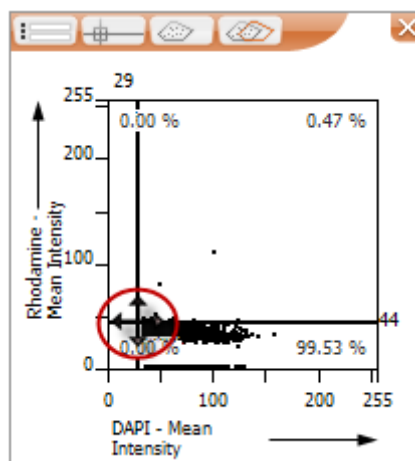


Figure 87 - Copy cutoff: step 1

- Click on the cutoff using the right mouse button, to see the contextual menu. Select the **Copy cutoff** entry in the menu. The cutoff in the scattergram is copied and can be pasted into another scattergram.

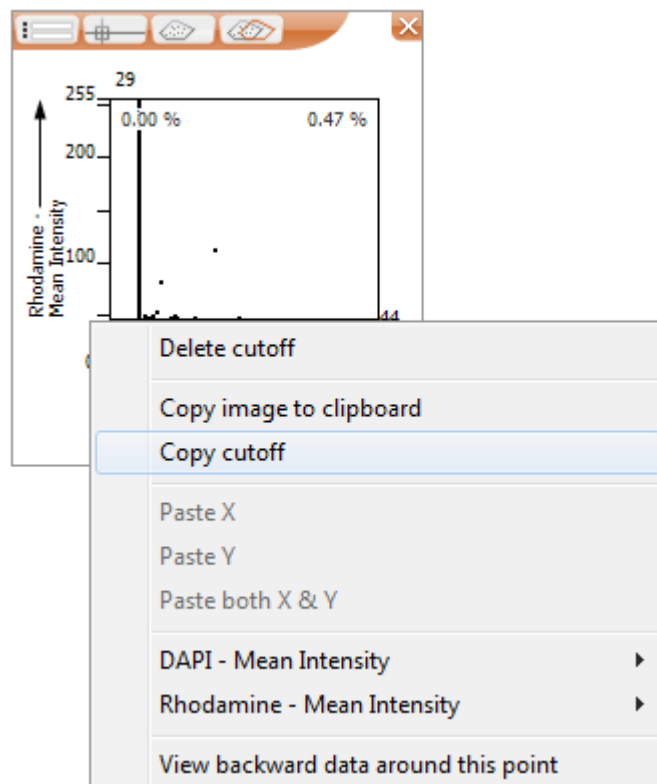
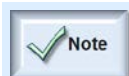


Figure 88 - Copy cutoff: step 2

10.4.5 Paste cutoff



- If you want to paste a cutoff, it must be already copied. To copy a cutoff, see **Chapter 10.4.4.**

- Move the mouse cursor inside the diagram area of the scattergram where you want to paste the cutoff and right click to see the contextual menu.
- Select the **Paste both X&Y** if you want to paste both cutoffs (horizontal and vertical); select **Paste X** (only the vertical cutoff); select **Paste Y** (only the horizontal cutoff).

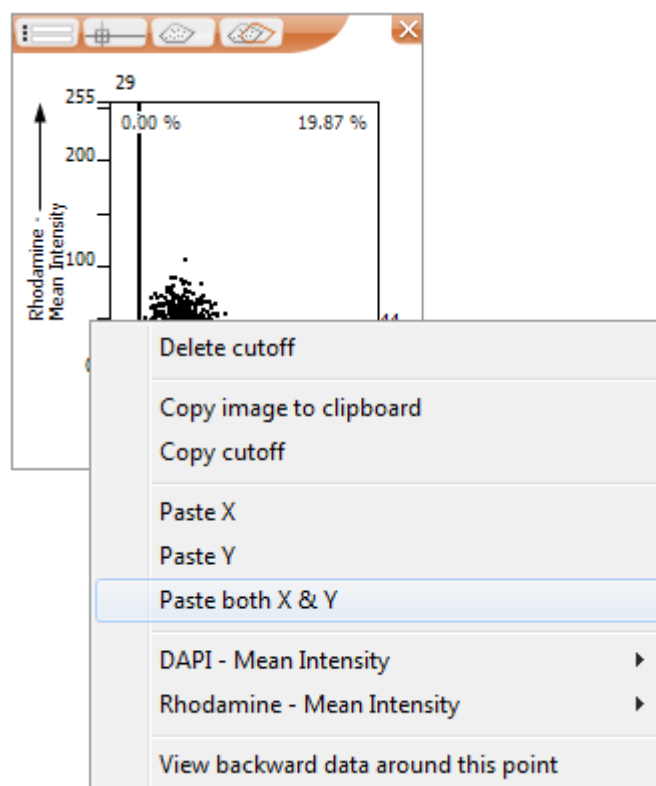


Figure 89 - Paste cutoff



- The cutoff immediately appears in the scattergram.

10.5. Set gates

10.5.1 Add rectangular gate



- If you add gates before adding the cutoffs, the statistics of the gates will not appear.

- Click **Add gate** button () in the scattergram toolbar.
- After clicking on the button, move the mouse into the chart area of the scattergram. The cursor changes into a cross ().

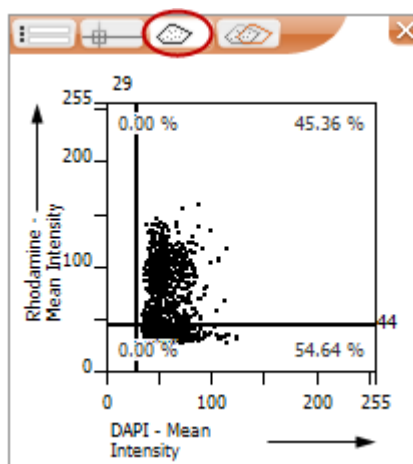


Figure 90 - Add gate: step 1

- Press the left mouse button inside the chart area to define the starting point of the region of interest.
- Holding the left mouse button pressed, resize the rectangular region to the desired size.

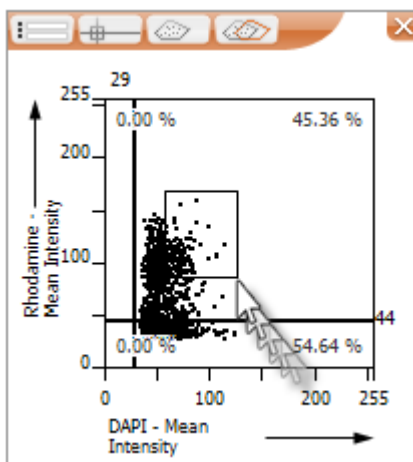


Figure 91 - Add gate: step 2

- Release the left mouse button to finally determine the gate. All the events inside the gate will change their color to a predefined hue. The gate is labeled with a default text (e.g. "Gate 1"). The color, the label and the visibility of each gate can be modified in the gate management (see [Chapter 10.5.5](#)).

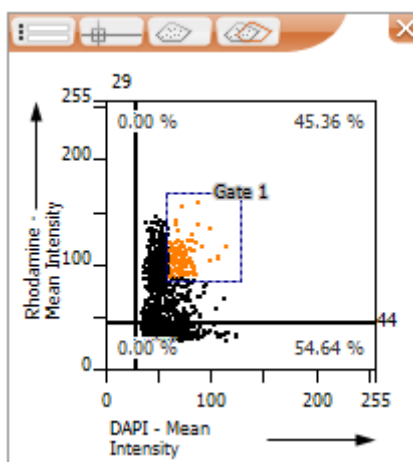


Figure 92 - Add gate: step 3

10.5.2 Polygon gating method

- Click on the **Add gate** button () in the scattergram toolbar.

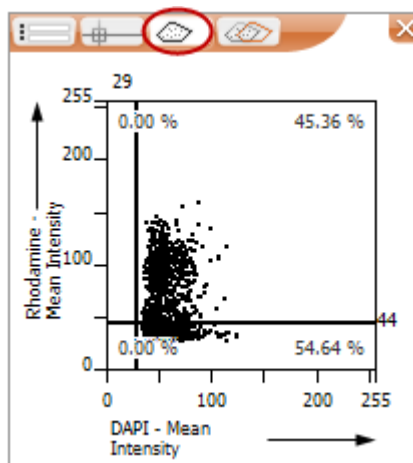


Figure 93 - Add polygonal gate: step 1

- After clicking on the button, move the mouse into the chart area of the scattergram. The cursor changes into a cross (\times).
- Click the left mouse button inside the chart area to define the starting point of the region of interest.
- Now, a red line appears and connects the last added point with the current mouse position.
- To define the next point of the polygon, click the left mouse button in the chart area. The red line continues to connect the last added point with the current mouse position.

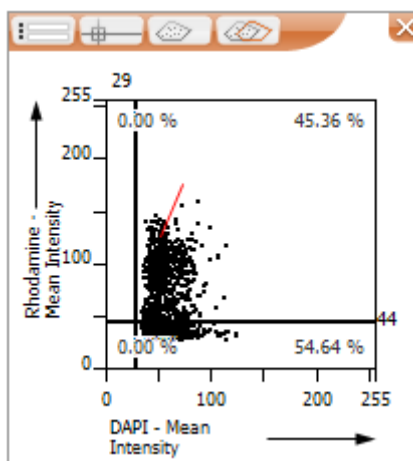


Figure 94 - Add polygonal gate: step 2

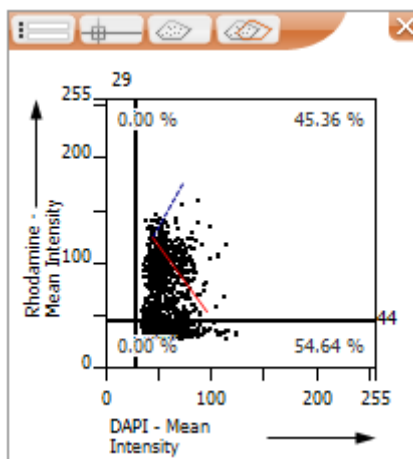


Figure 95 - Add polygonal gate: step 3

- Repeat the last two steps until all the points are defined;
- The last step in setting a polygonal gate is to close the polygon. To do this, click the right mouse button inside the chart area. This will define the last point of the polygon and will connect the last point to the first point. All events inside the gate change their color to a predefined hue. The gate gets labeled with a default text (e.g. "Gate 1"). You can change the color, the label and the visibility of each gate by using the gate management (see [Chapter 10.5.5](#)).

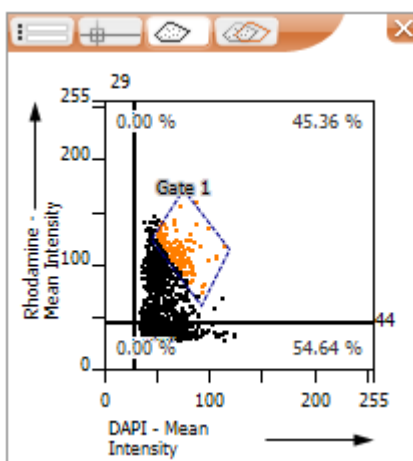


Figure 96 - Add polygonal gate: step 4

10.5.3 Modify gate

- Click the **Edit gate button** () in the scattergram toolbar.

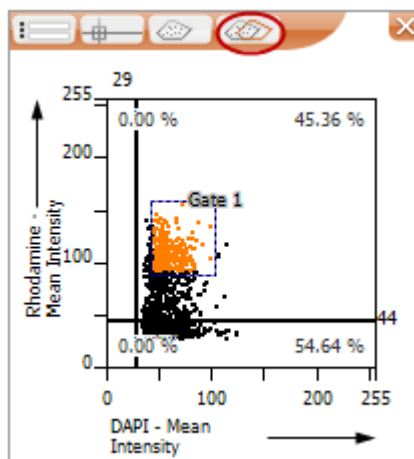


Figure 97 - Modify gate: step 1

- The corner points of the gates are highlighted by a small rectangle, showing the gate is ready to be moved.

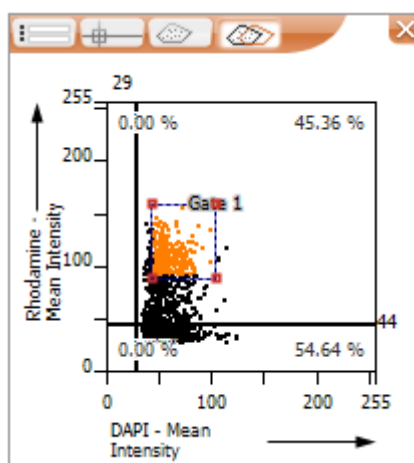


Figure 98 - Modify gate: step 2

- Move the cursor over the corner that you want to move. The cursor changes into a cross (\oplus).
- Press the left mouse button, hold it pressed and move the point to the new position. During the movement, the new shape of the gate is highlighted in red lines.

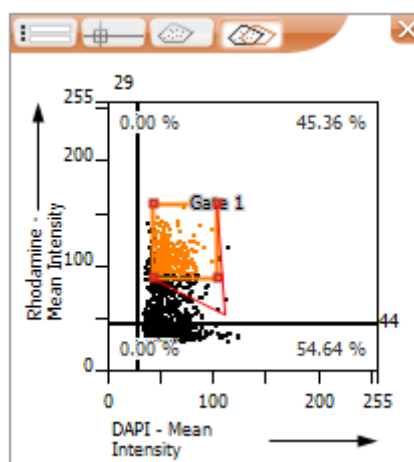


Figure 99 - Modify gate: step 3

- When the new shape of the gate meets your needs, release the left mouse button.

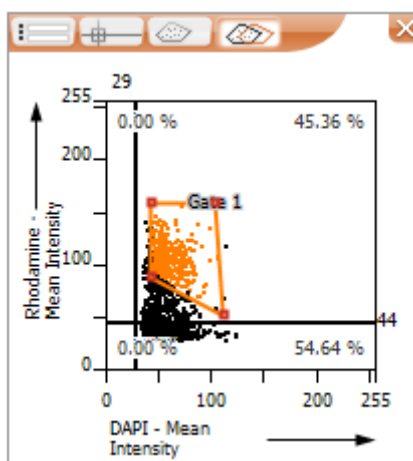


Figure 100 - Modify gate: step 4 (a)

- This procedure can be repeated for every corner of the gate.

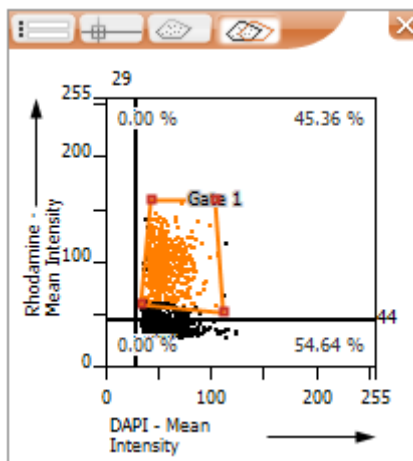



Figure 101 - Modify gate: step 4 (b)

- After finishing the modifications, press again the **Edit gate button** () to disable the function.

10.5.4 Delete gate

- Click the **Edit gate button** () in the scattergram toolbar.

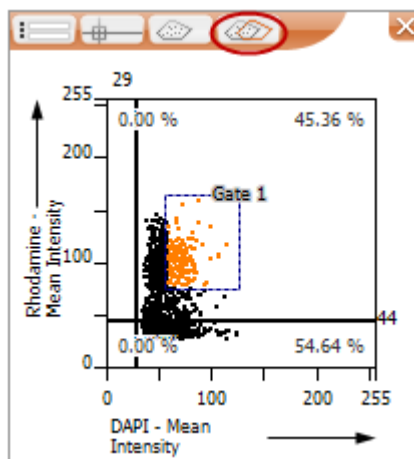


Figure 102 - Delete gate: step 1

- The corner points of the gate are highlighted by a small red rectangle.

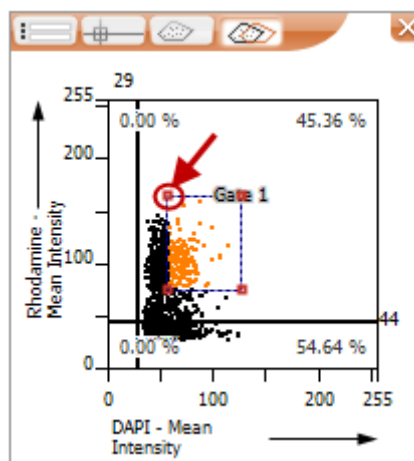


Figure 103 - Delete gate: step 2

- Select the gate that you want to delete by clicking one of its corner points. In this case it doesn't matter which of the corner points you choose for selection. When the gate is selected, the lines are highlighted.

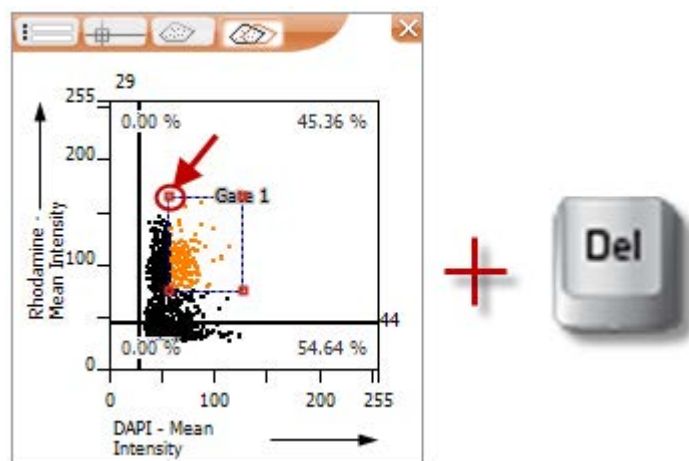
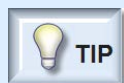


Figure 104 - Delete gate: step 3

- Press **Delete** on your keyboard. The selected gate will immediately disappear.



- You can also delete a gate from **Gate management**. See [Chapter 10.5.5](#).

10.5.5 Gate Management

To delete gates or to modify the gates display settings, select **Gates** → **Manage...** from the application's main menu.



Figure 105 - Gate management menu

The **Gate management** dialog will appear where you can do the following operations:

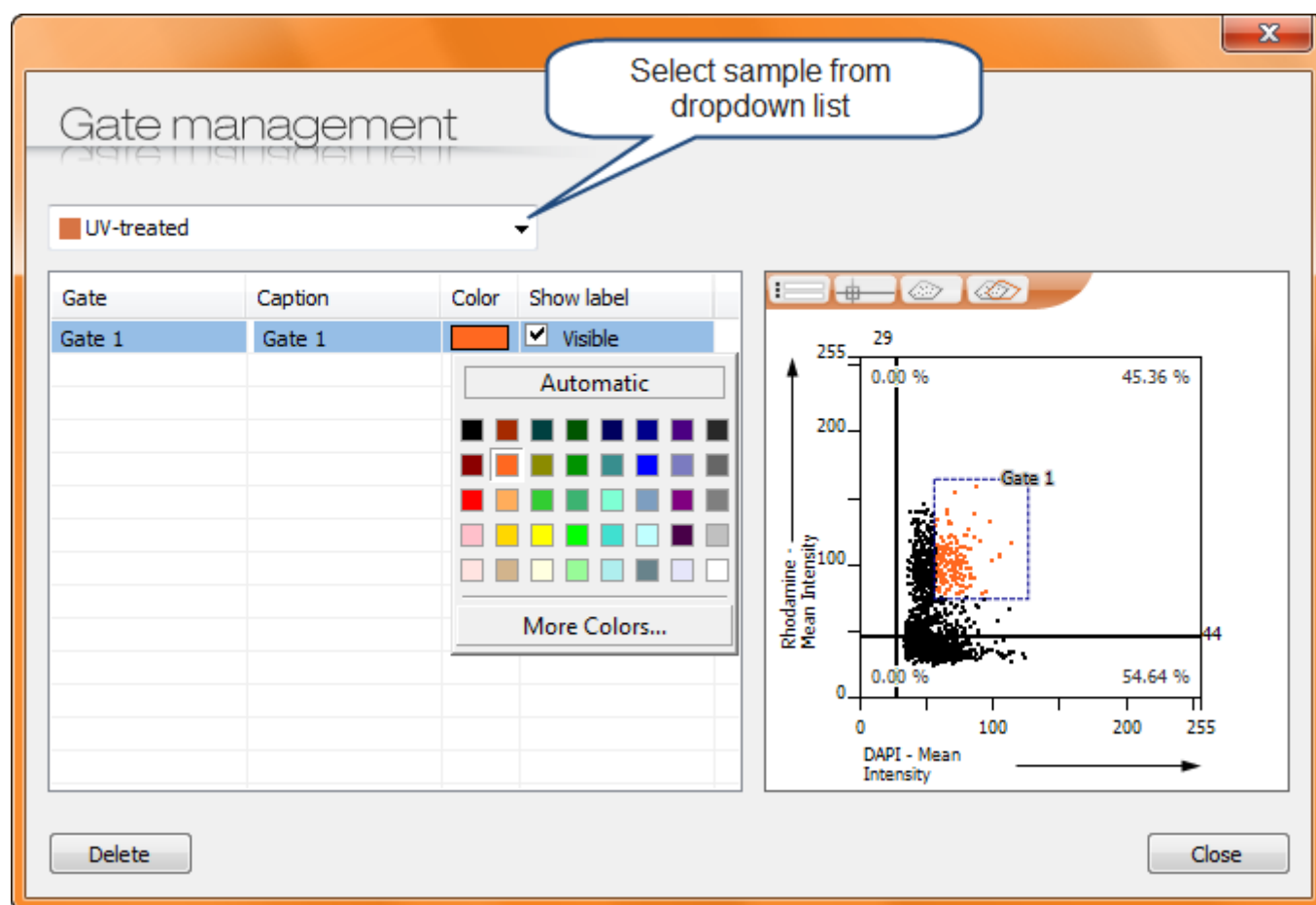


Figure 106 - Gate management dialog

- Select desired sample from dropdown list, by pressing the  button.

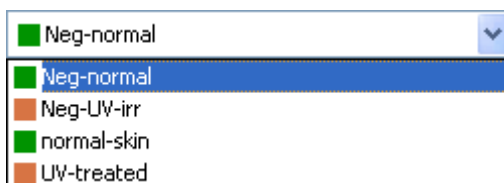


Figure 107 - Gate management: select sample

- By default, every gate has a name (for example *Gate 1*, *Gate 2*). You can change this name by double clicking on the actual name of the gate in the **Caption** field and typing the desired new name.

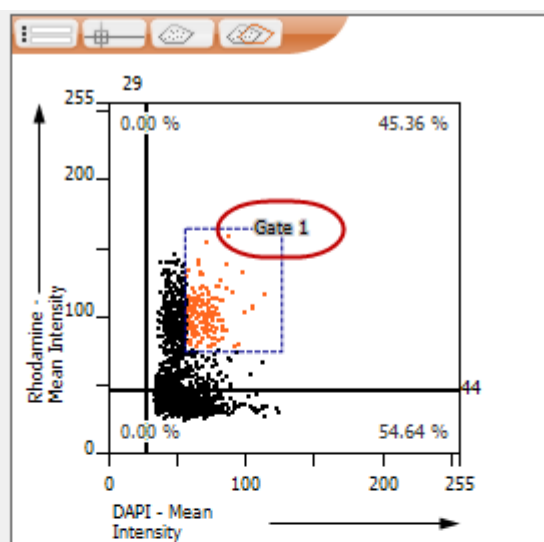
Gate	Caption	Color	Show label
Gate 1	Gate 1		<input checked="" type="checkbox"/> Visible

Figure 108 - Gate management: rename gate

- You can set any color for the events inside the gate by double clicking on the **Color** field of the desired gate and choose a color from the color picker.
- You can display or hide the name of the gate on the scattergram by checking / unchecking **Show label** option for the desired gate.

Gate	Caption	Color	Show label
Gate 1	Gate 1		<input checked="" type="checkbox"/> Visible

1



Gate	Caption	Color	Show label
Gate 1	Gate 1		<input type="checkbox"/> Hidden

2

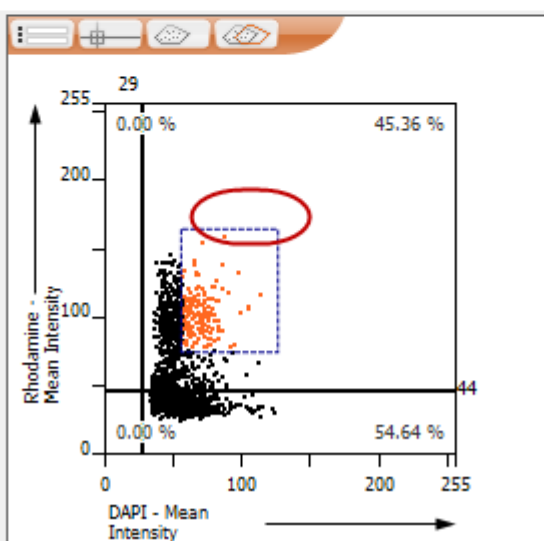


Figure 109 - Gate management: Show label

- To delete a gate from the list, select it by left clicking on it, then press **Delete** button ().

10.6. Scattergram parameters

By right clicking on the scattergram, a contextual menu will appear. From this contextual menu, the scattergram parameters can be adjusted.

For each scattergram parameter, the following properties can be adjusted:

- Autoscale
- Maximum value (if the Autoscale is not used)
- Scaling type

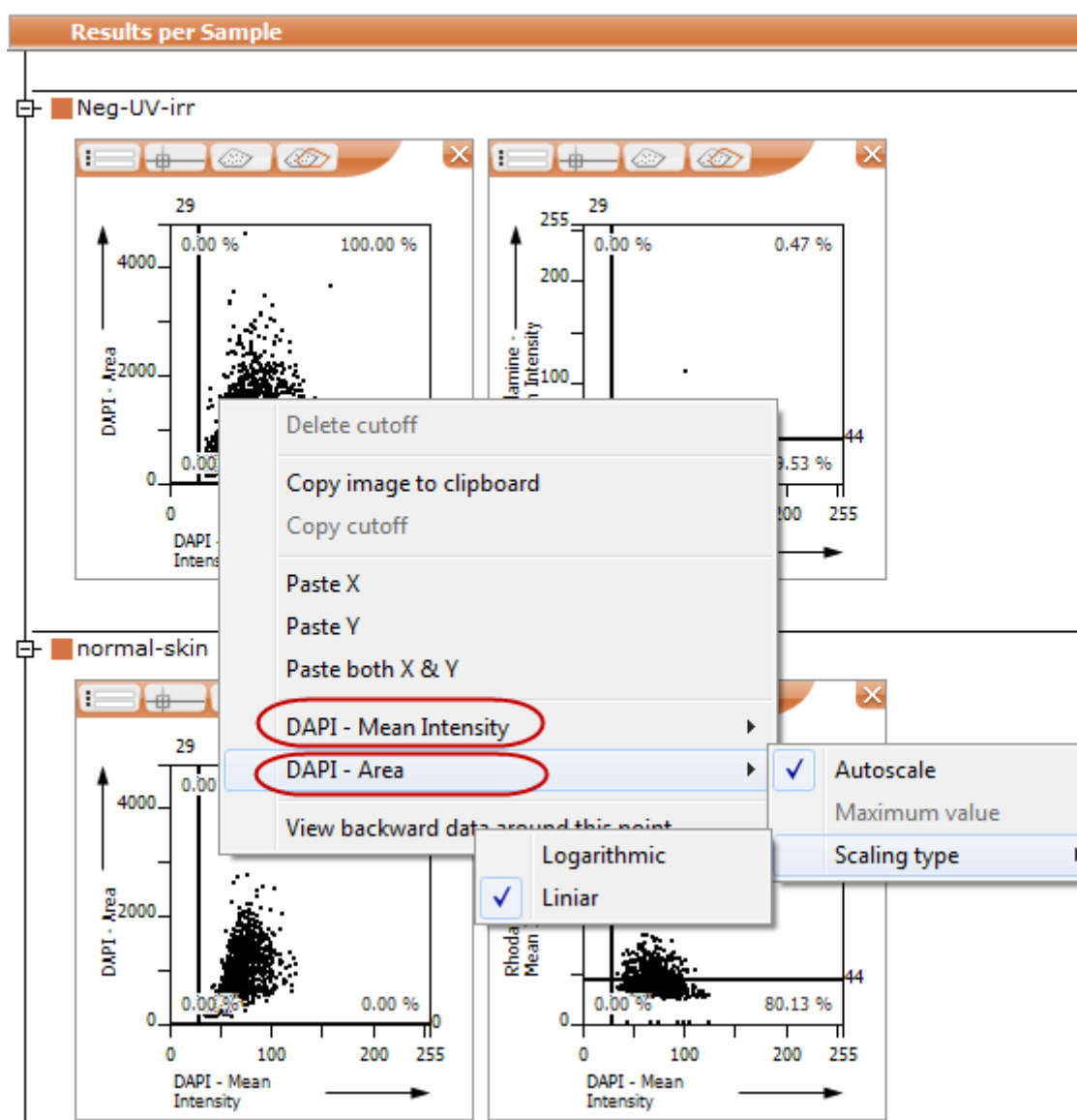


Figure 110 - Scattergram parameters

10.7. View backward data

- By right clicking on the border of a gate, a contextual menu will appear: View backward data for gate "Gate 1"
If you select **View backward data for gate [Gate Name]**, you will see the backward data for that gate. For details see [Chapter 11](#).

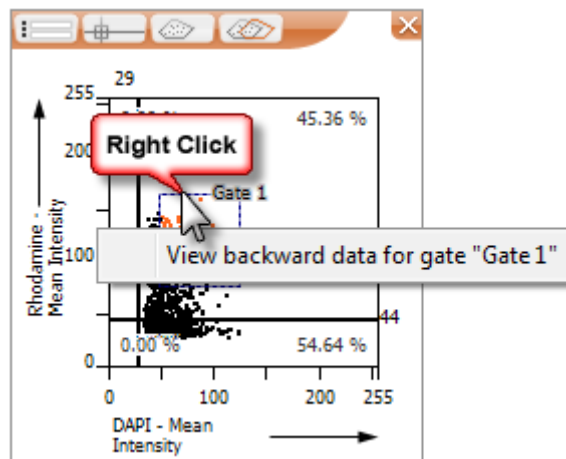


Figure 111 - View backward data for a gate

- By right clicking outside the border of a gate, a contextual menu as shown below will appear. If you select **View backward data around this point**, you will see the backward data for that numerical value. For details see [Chapter 11](#).

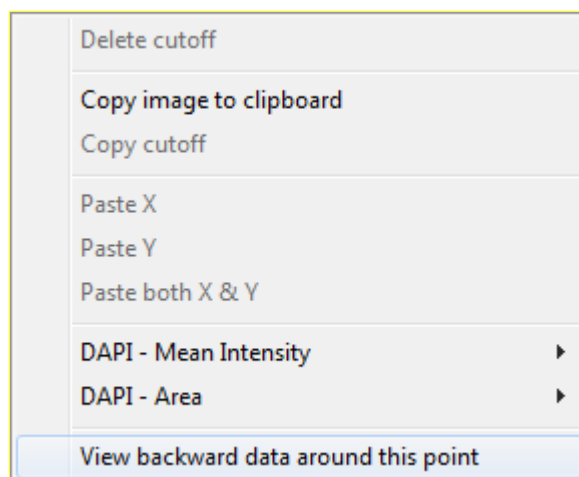


Figure 112 - Scattergrams: contextual menu

11. Forward and backward connection

One of the most fascinating and instructive features of **TissueFAXS** is the ability to connect image objects with data space, i.e. the user can visualize any particular cell of an image in the scattergram and vice versa!

11.1. Forward connection

The **forward connection** is a feature that allows you to access the numerical results for a specific event.

To view the respective data, follow the next steps:

- In the **Detail Window** that displays the image of a channel, just double-click on an event. That event will be highlighted by a red and white mark like a little flashing square.

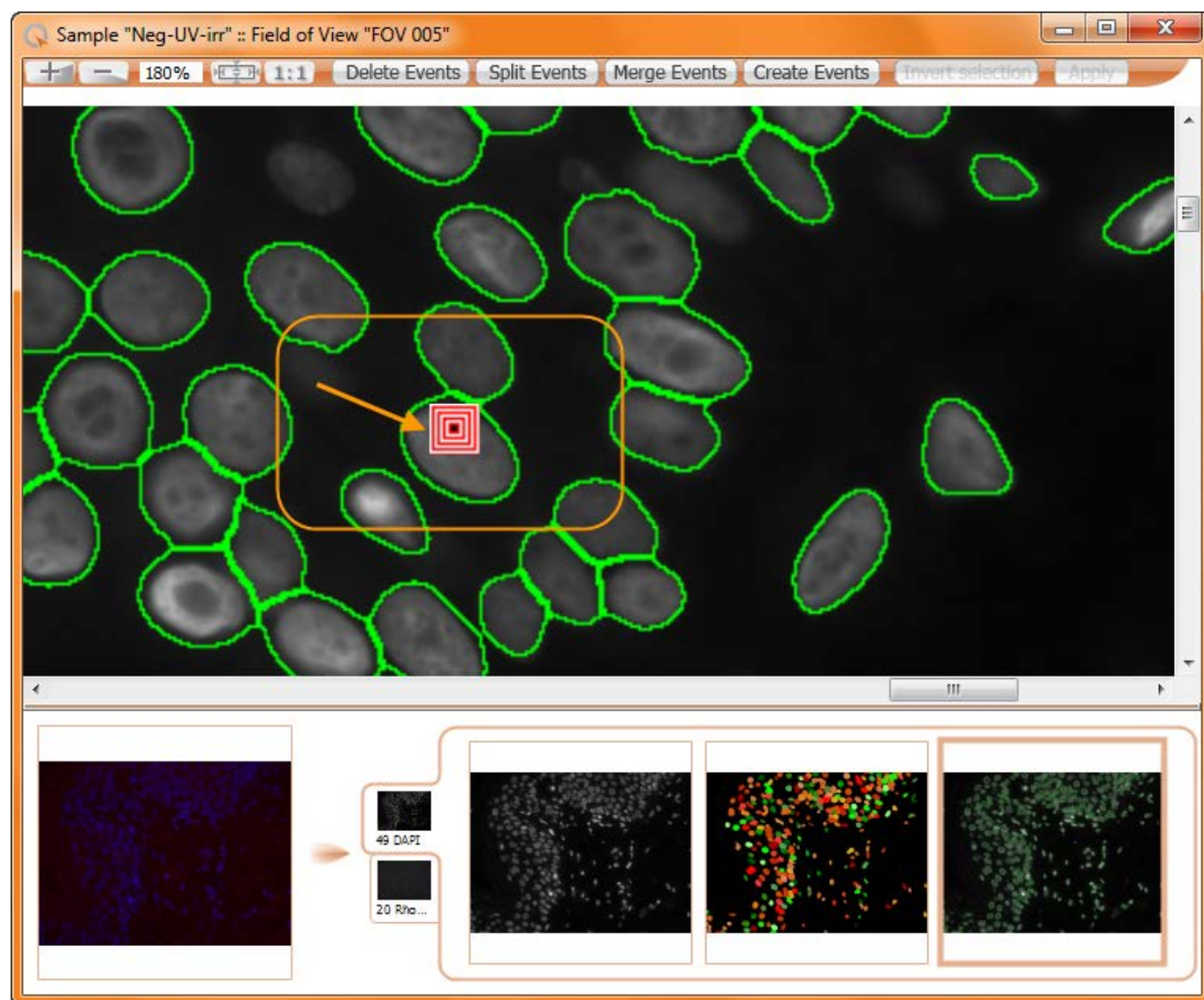


Figure 113 - Forward connection: step 1

- In all the **scattergrams** corresponding to the sample, the results will be highlighted by a red and black mark like a little flashing square.

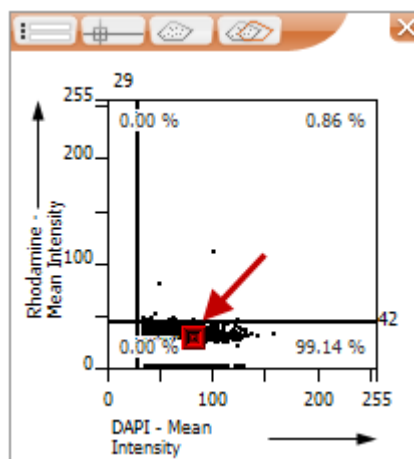


Figure 114 - Forward connection: step 2

11.2. Backward connection

The **backward connection** is a feature that allows you to access the events that have specific numerical results. Backward connection can be used for a single numerical value or for an entire gate.

To view the events corresponding to a **single numerical value**, follow the next steps:

- Within a scattergram, right click on a dot.
- In the context menu that appears, choose **View backward data around this point**.

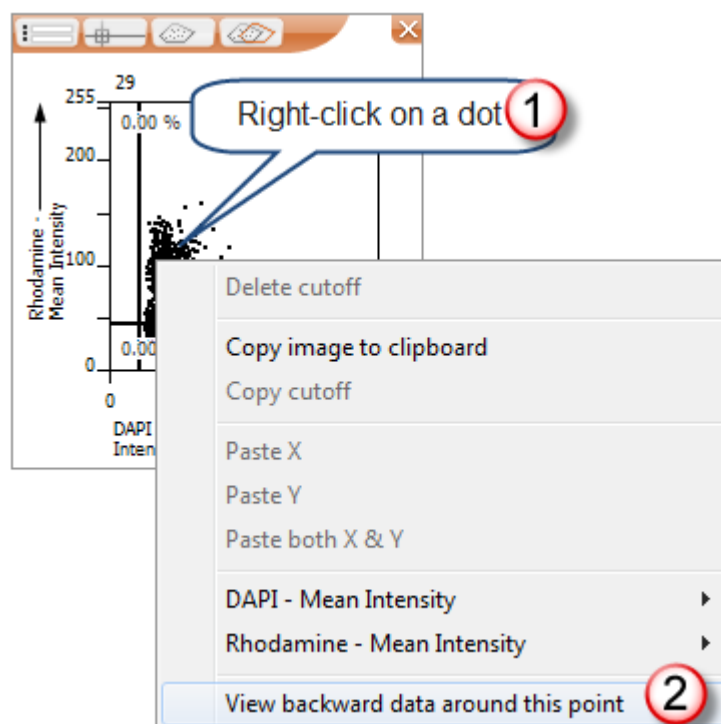


Figure 115 - Backward connection: single numerical value (a)

- After backward data is processed, the corresponding events can be followed up in **Detail window**.

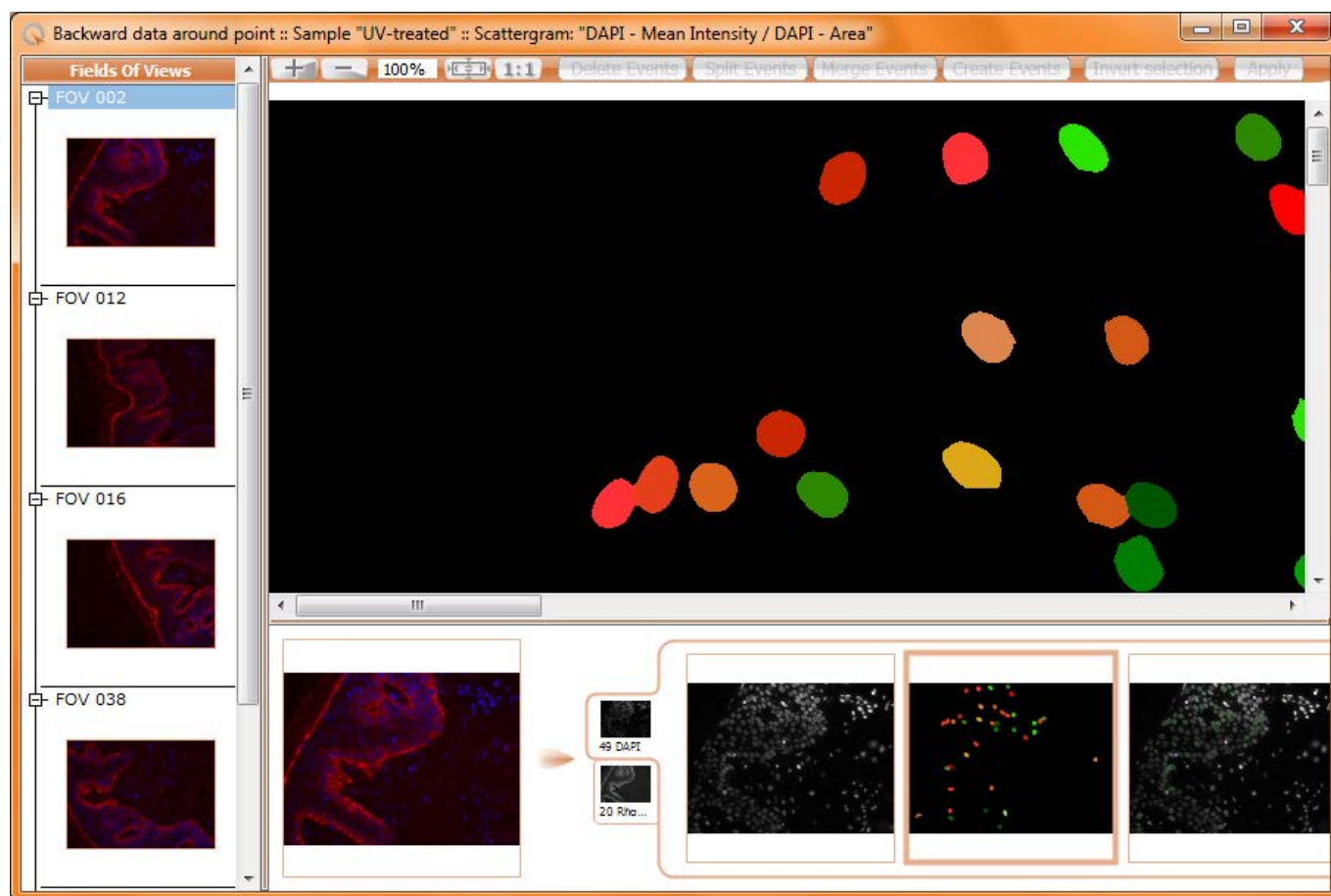


Figure 116 - Backward connection: single numerical value (b)

To check the events corresponding to **an entire gate**, follow the next steps:

- Within a scattergram, right click on the border of a gate.
- In the context menu that appears, choose **View backward data for [Gate name]**.

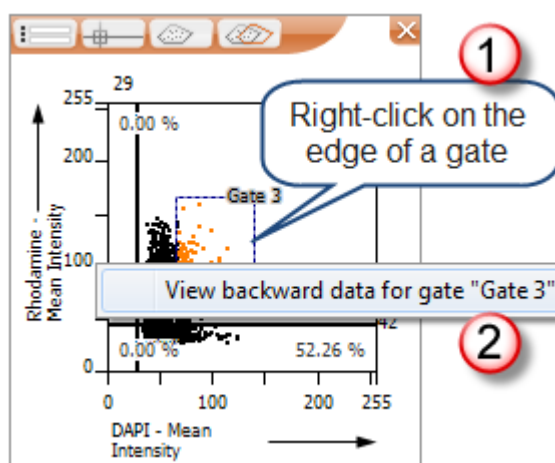


Figure 117 - Backward connection: gate (a)

- After backward data is processed, the corresponding events can be followed up in **Detail window**.

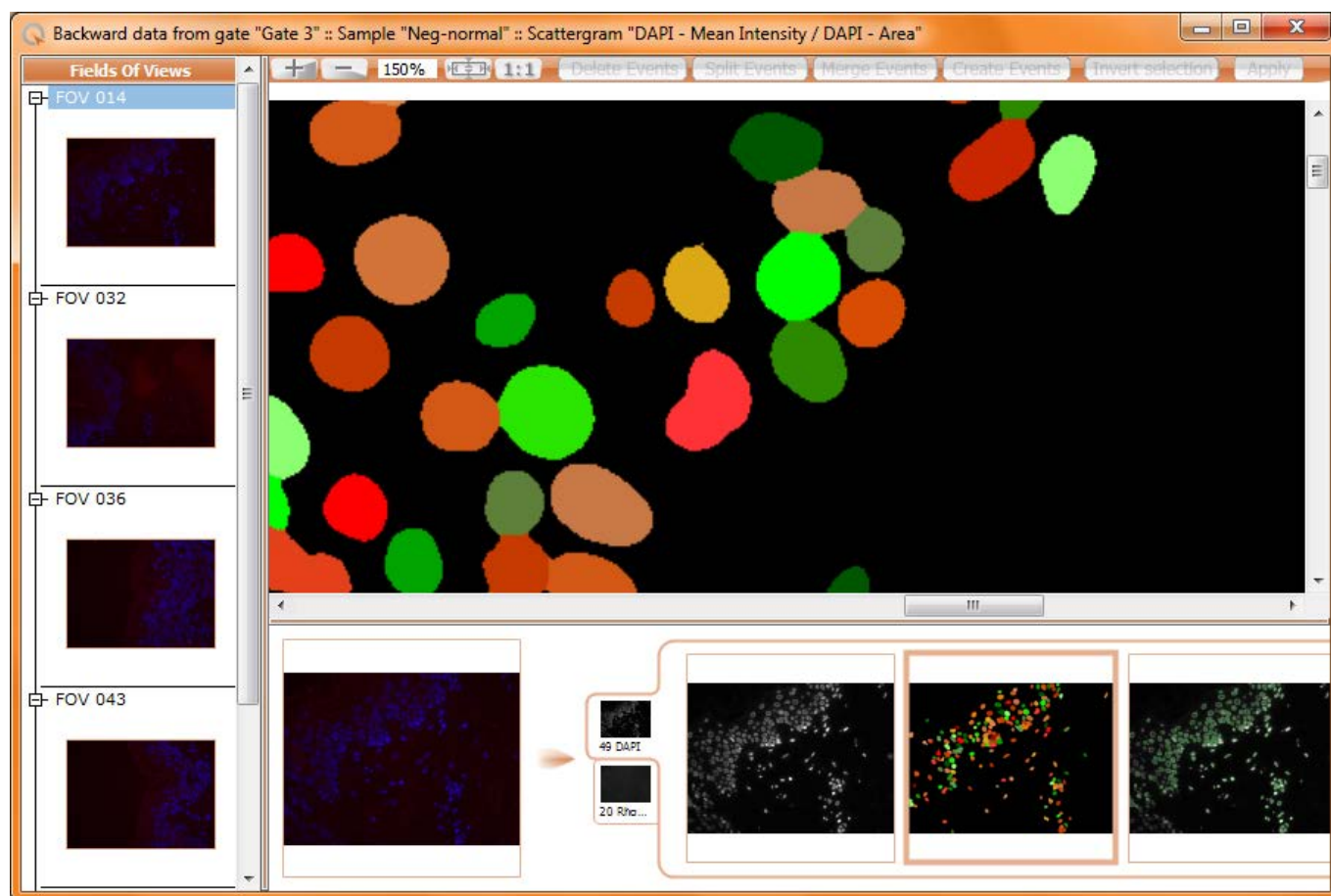


Figure 118 - Backward connection: gate (b)

12. Project properties

To access **Project properties**, go to the **Menu** toolbar and choose **Project** → **Properties...**

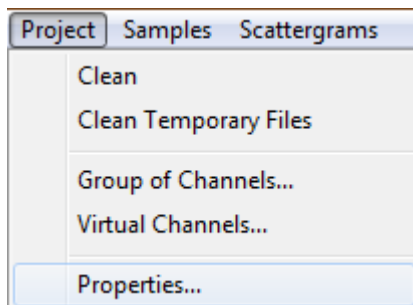


Figure 119 - Project Properties menu

The **Project Properties** window will appear:

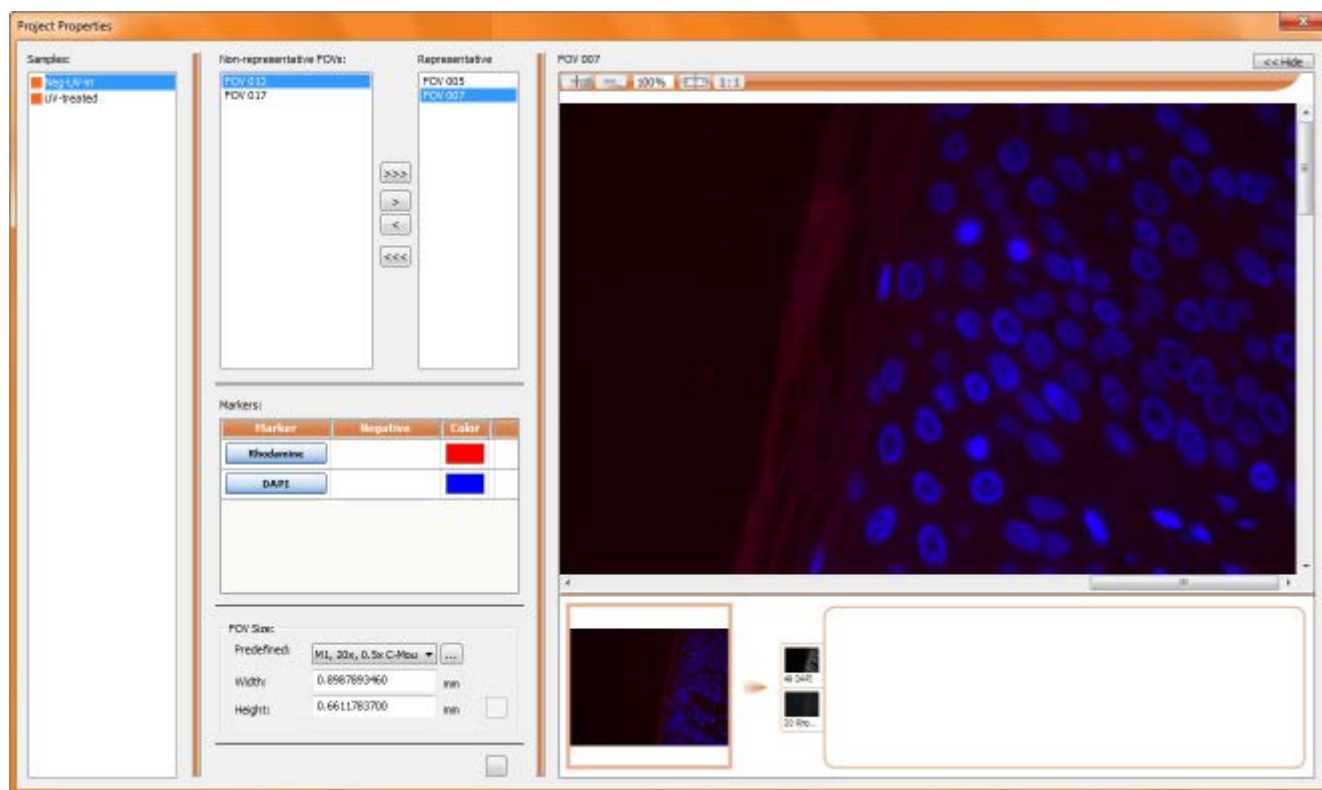


Figure 120 - Project Properties window



- To hide the overview of the FOV and of the channels, press **Hide** button (<< Hide) in the upper-right corner of the window.

12.1. Samples

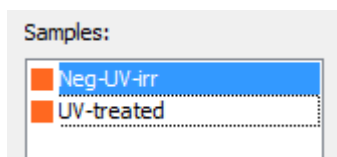


Figure 121 - Project Properties: Samples section

In the **Samples** section, you can choose for what samples you want to adjust settings. Simply click on the desired sample to make it available.

12.2. Representative/Non-representative FOVs

Even with modern computer technology, image analysis is a time consuming task. For optimizing the settings for image segmentation, certain fields of view can be declared as “representative fields of view”. This allows faster evaluation of certain settings. Once the settings have been defined on the representative FOVs, the analysis can be run on the entire samples/all FOVs in the project.

Representatives FOVs are FOVs that include a significant amount of information in order to generate concluding results. The results of the analysis of the representative FOVs should be similar to the analysis of all FOVs.

You can move your FOVs from one list to another according to your needs.

In this section you will learn how to select your representative FOVs. Two lists of FOVs are available:

- Non-representative FOVs (to the left)
- Representative FOVS (to the right)

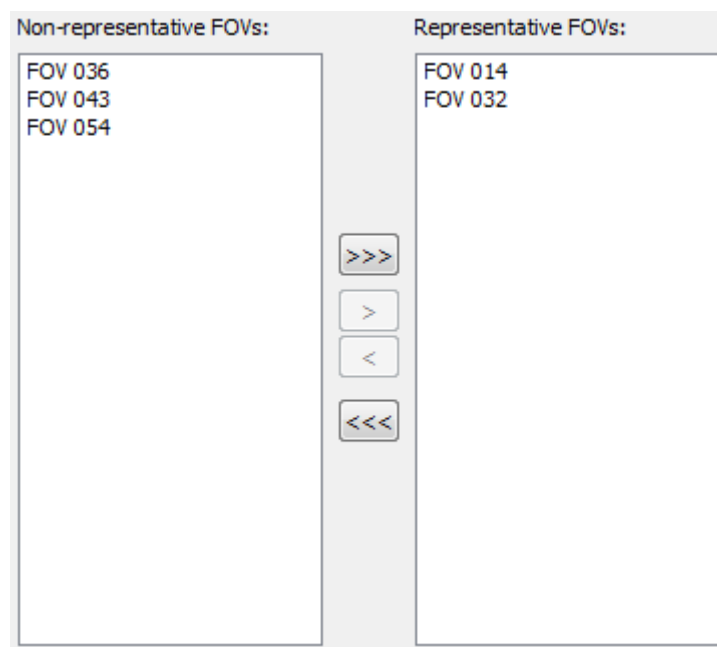



Figure 122 - Project Properties: Representative/Non-representative FOVs section

Moving one or more FOVs

- To move one or more FOV from the **Non-representative FOVs** list to the **Representative FOVs** list, select the FOVs you want to move (by clicking on them - hold **Control** key pressed for multiple selection), then press the  button.
- The FOVs will immediately appear in the **Representative FOVs** list.
- To send the FOVs back to the **Non-representative FOVs** list, select the desired FOVs and then press the  button.

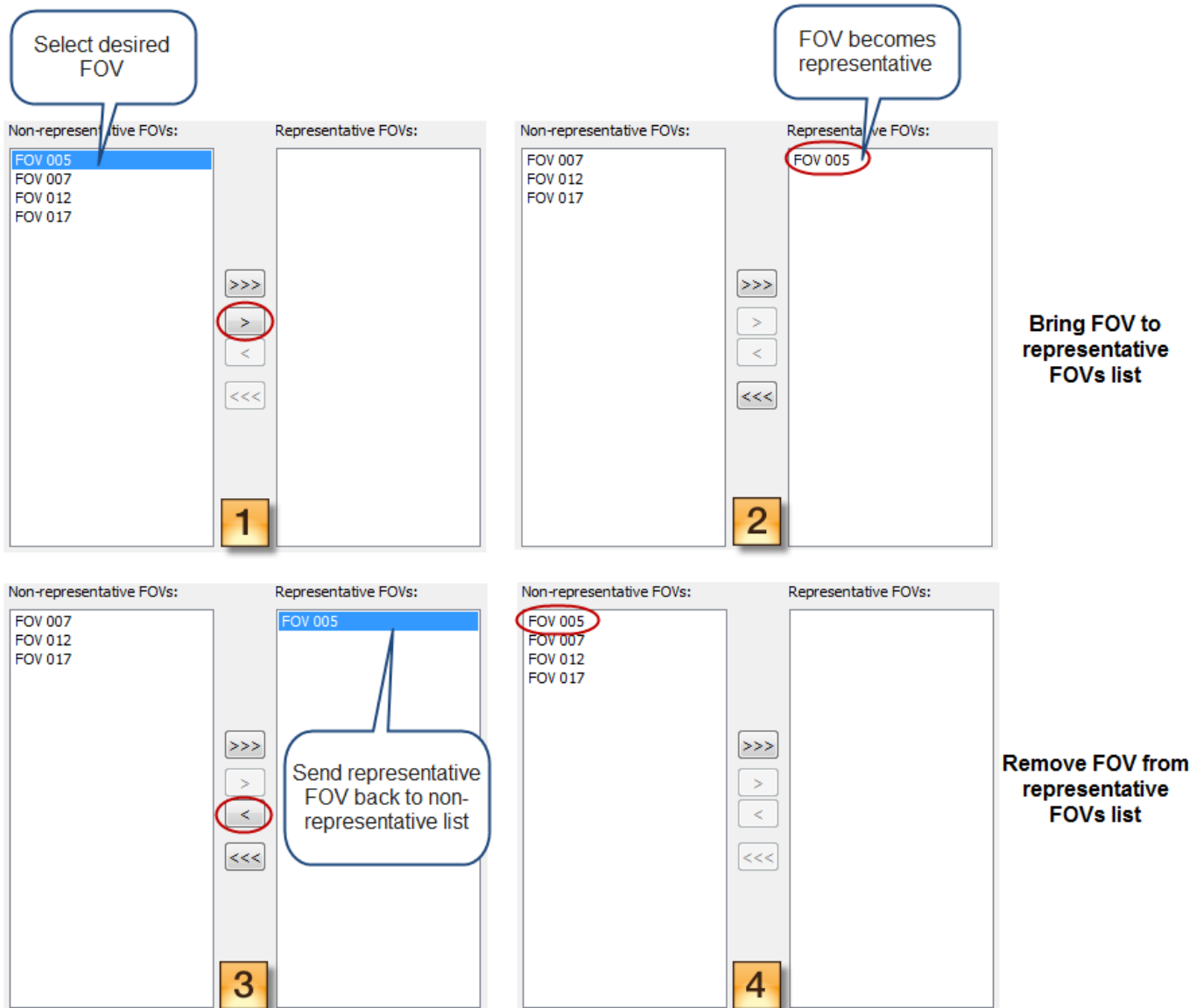


Figure 123 - Project Properties: Representative/Non-representative FOVs - moving one FOV

Moving all FOVs

- To move all FOVs from the **Non-representative FOVs** list to the **Representative FOVs** list, press the  button.
- The FOVs will immediately appear in the **Representative FOVs** list.
- To send all FOVs back to the **Non-representative FOVs** list, press the  button.

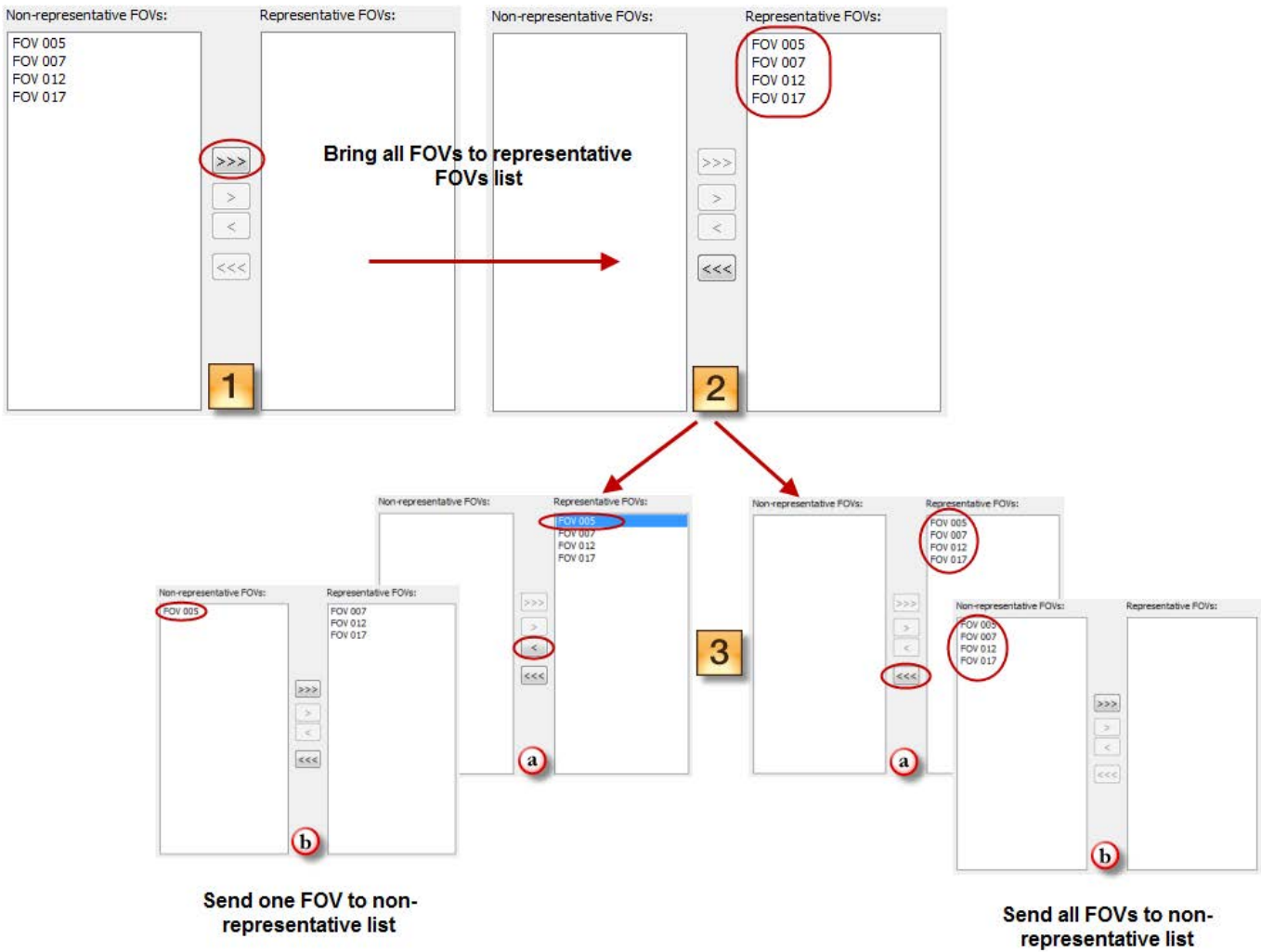


Figure 124 - Project Properties: Representative/Non-representative FOVs - moving all FOVs

12.3. Markers

In this section are listed all the **markers** (i.e. antibody staining, chemical reagent, type of molecule visualized by means of immunofluorescence) in the project and you can change their settings.

Markers:				
Marker	Negative	Color	Master	
Rhodamine			<input type="checkbox"/>	
DAPI			<input checked="" type="checkbox"/>	

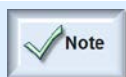
Figure 125 - Project Properties: Markers section

- You can select a color for each marker by clicking in its corresponding **Color** area (see picture below).



Figure 126 - Project Properties: choosing marker color

- You can choose the master marker by marking the corresponding **Master** checkbox of the desired marker.



- If you change the master channel, all results belonging to the affected samples will be lost (including mask and overlay images, numerical results, cutoff and gates).

12.4. FOV Size

In this section you can set the dimensions of the FOVs. You can do this in two ways:

Predefined option

Use the **Predefined** option by choosing an existing template from the dropdown menu.

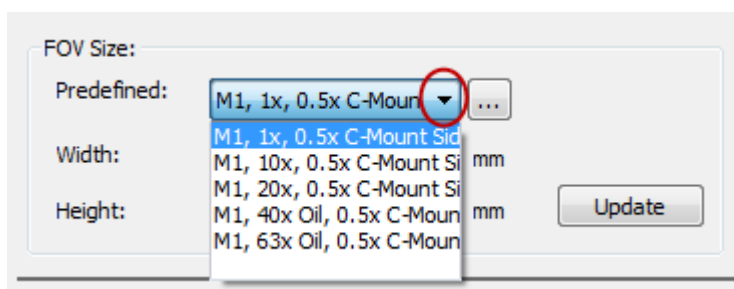

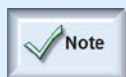


Figure 127 - Project Properties: FOV Size section

- By pressing the  button, the **Templates for FOV' Size** dialog will appear.
- In this dialog you can see details about the existing templates or create a new template.
- You can also **modify a template** by changing its name, description and the Width and Height values of the FOV.
- To **create a new template** you must specify a name, a description and the Width and Height values of the FOV (in *mm*).
- You can also **delete** a template: first select it in the list (by clicking on it), then press **Remove** button.
- After each operation, don't forget pressing the **Update** button.



- If wrong dimensions are used, the computed cell densities (i.e. cells per mm^2) will be **WRONG!** Other parameters as well as original measurement values are not affected!

- For more details concerning Templates for FOV' size, also see **Chapter 4.4.1**.

Manually adjust Width and Height values of the FOV

To manually edit **Width** and **Height** values of the FOV (the “scaling” or image dimension), simply type the desired values in the corresponding fields.

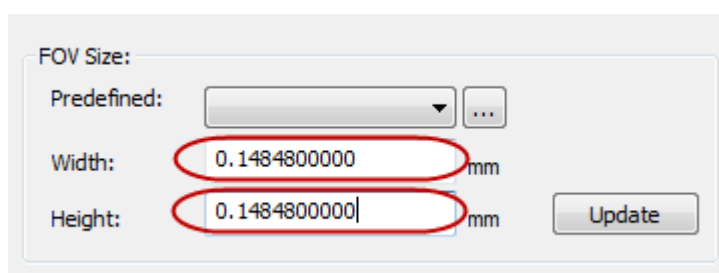


Figure 128 - Project Properties: manually edit Width and Height values

After adjusting the size of the FOV, press **Update** button.

13. Evaluation

13.1. Experiment evaluation

Evaluation is realized on two levels. During the process of setting the parameter for the pattern recognition, the results obtained by those image processing procedures have to be optically evaluated for principal adequacy.

After the image analysis process is accomplished, the obtained data need to be evaluated by appropriately setting cutoff values based on controls, and/or setting gates in order to define subpopulations. The quadrant statistics contains the statistical evaluation for each of the four quadrants in each scattergram.

13.2. Scattergram evaluation

The **scattergram** shows two parameters out of the measurement data set. They can be shown on a linear (e.g. DNA staining intensity) or a logarithmic (e.g. mean relative fluorescence of antibodies) scale. Depending on what the user is investigating, new scattergrams may be created at any time and the parameters on one or both axes of existing scattergrams may be changed. Existing scattergrams may be hidden or deleted (see **Chapter 10.1.3**).

Each point in a scattergram represents a single measure event (nucleus, cell, cellular compartment or any other structure, depending on the respective marker used for labeling in that channel). In other words, each point represents the correlation of two parameters of a single measure event.

For each parameter that is represented on each axis, a **cutoff** value may be established in order to define the level of specificity for each marker (i.e. what is a specific staining and what is background?). Single cells with a specific staining reactivity for a given marker are referred to as “positive for that marker”. Cells lacking such a specific staining are referred to as “negative for that marker”.

The exact value for each cutoff has to be determined by appropriate controls (e.g. isotype-matched negative controls for antibody-based labeling). These two cutoffs per scattergram define four quadrant regions (LL – lower left, UL – upper left, UR – upper right and LR – lower right), which may contain different populations of measured events in the respective scattergram:

- LL: Marker X negative / Marker Y negative
- UL: Marker X negative / Marker Y positive
- UR: Marker X positive / Marker Y positive
- LR: Marker X positive / Marker Y negative

Beyond the basic distinction of positive and negative event populations, there may be less obvious differences in the samples analyzed. In order to acknowledge these differences, the **gate** tool may be applied (see **Chapter 10.5**). Gates allow distinguishing between positive populations (e.g. all measure events above a certain cutoff value) that are low, intermediate or high for a given marker. The advantage is that the result of this procedure is an exact measurement with full statistical information, rather than a rough estimation as obtainable by just visual evaluation.

The quadrant statistics provides information about the mean x- and y- labeling intensity, about the absolute and relative number of events per quadrant, and – if the user can provide information about the scales in the microscope used to acquire the images – also the number of events per mm².

14. Special features

14.1. Print project

To **print the project** press **Print current project** button ().

The following dialog will appear:

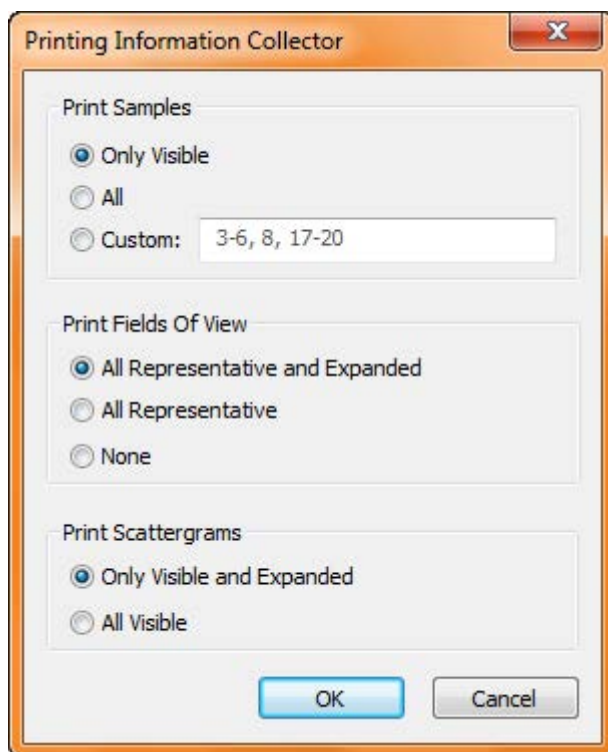


Figure 129 - Printing Information Collector

In the **Printing Information Collector** dialog you can make the following settings:

- For **Samples**: you can print only visible samples, all samples or only the samples you want to select.
- For **Fields of View**: you can print all representatives and expanded FOVs, all representative FOVs or none.
- For **Scattergrams**: you can print only visible and expanded scattergrams or all visible scattergrams.

Before printing a project, you may want to see a **Print Preview**, available in **File** → **Print Preview....**

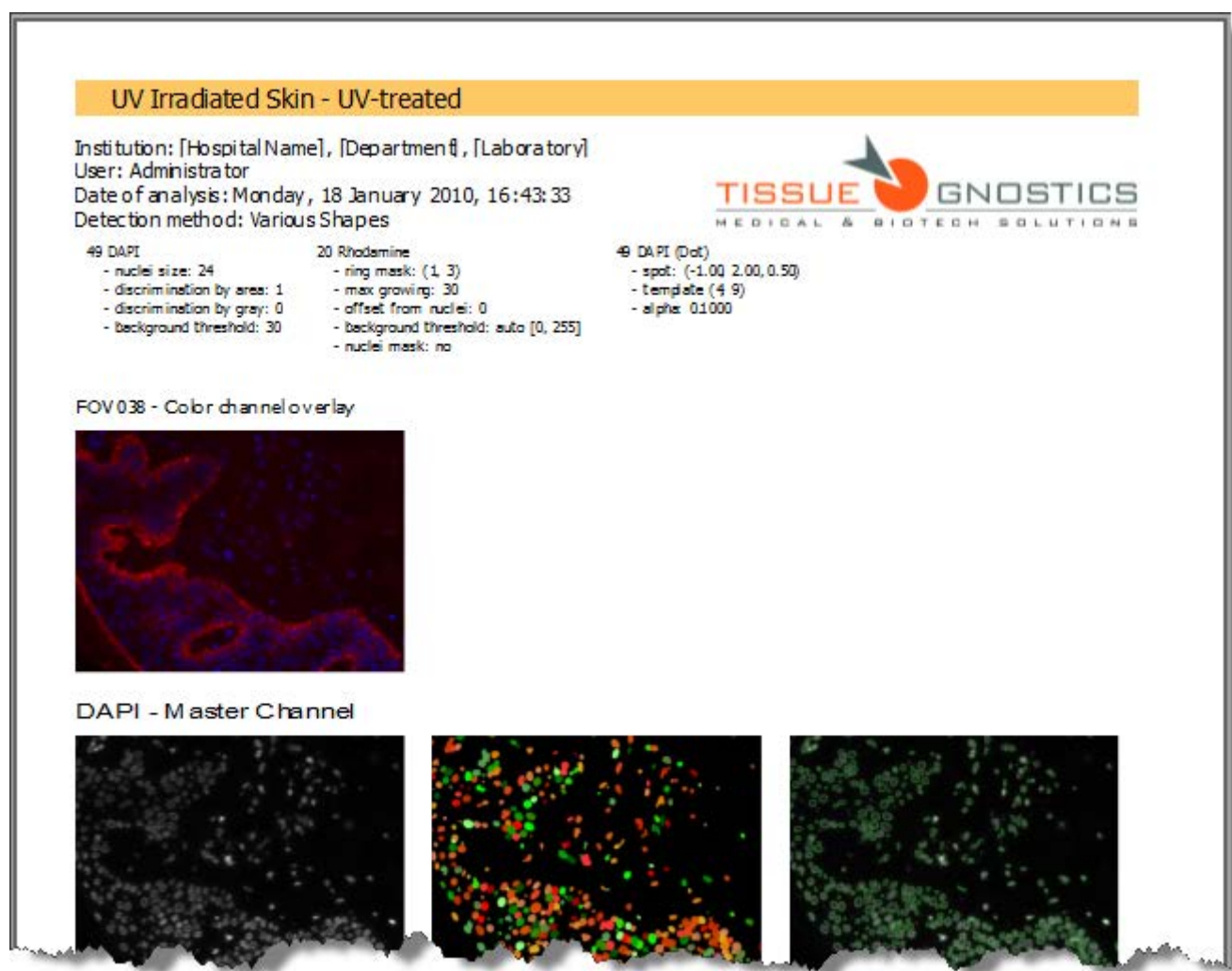


Figure 130 - Print Preview example

**TIP**

- You may also print a project by going to **File** menu of the application and choose **Print...**

14.2. Export

To access the export options in **TissueQuest**, select **File** → **Export** in the menu bar. A dropdown menu like in the image below will appear:

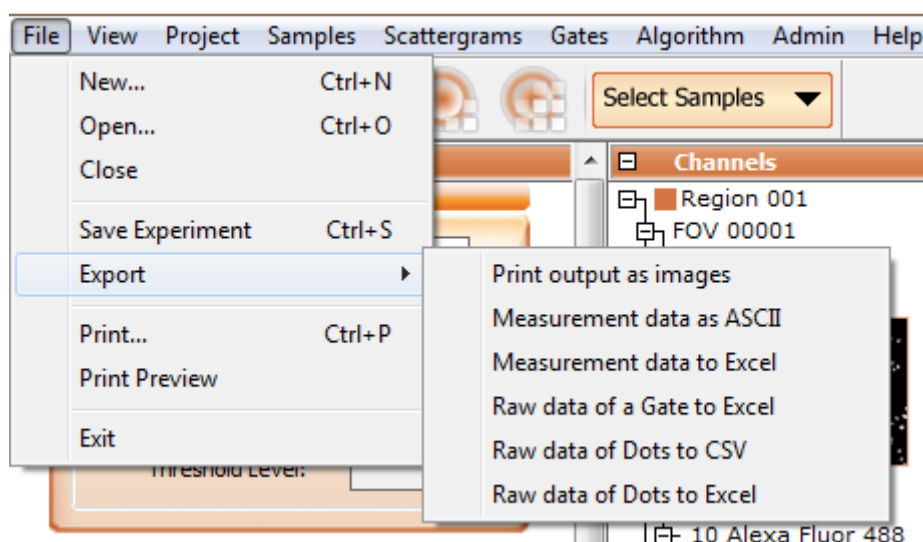


Figure 131 - Export project

The following options of exporting data are available:

- **Print output as images:** this feature allows you to save all the printing data as **images**, instead of printing it using a printer.
- **Measurement data as ASCII:** this feature allows you to export statistical data and raw data into **ASCII files** (text files).
- **Measurement data to Excel:** this feature allows you to export statistical data and raw data into **Excel files**.

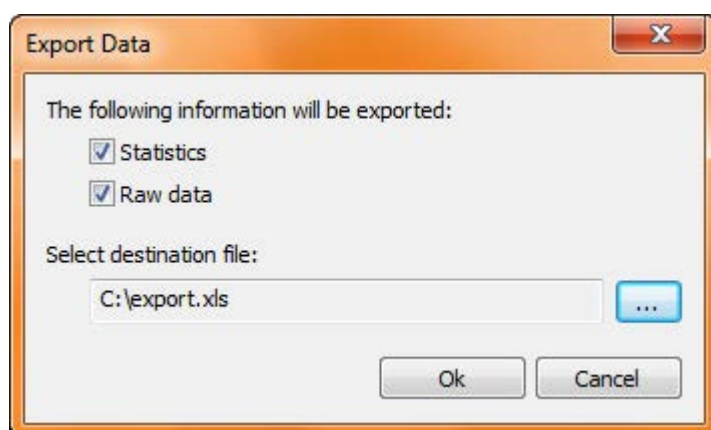


Figure 132 - Export Data to Excel

In **Exporting data to Excel**, you may choose what to include in export:

- **Statistics:** mark this checkbox if you want to include statistical data in export.
- **Raw data:** mark this checkbox if you want to include raw data in export.
- **Export Raw data of a Gate to Excel:** this option is available only for TissueQuest projects where all samples are linked, meaning the displayed/selected gates are propagated to all samples. If the samples are not all linked, this option will be disabled.
For each gate in each sample one or more sheets are created.
 - If all the events of the gate can be exported to only one sheet, its name will be "*GateName*"-"*SampleName*"-RawData.

- If the events of the gate can not be exported to only one sheet, its name will be "*GateName*"- "*SampleName*"-*RawData_*"%of%" ".

Each row corresponds to an event and it contains all parameters for all channels.

- **Export raw data of dots to CSV:** this option creates a .csv file for each sample and channel (named *SampleName-ChannelName.csv*). Each file exports the following data for dots (one dot per row):
 - Field Of View
 - Event ID
 - Dot ID
 - Dot Area
 - Dot Min Intensity
 - Dot Mean Intensity
 - Dot Max Intensity
 - Dot Sum Intensity
- **Export raw data of individual dots to excel:** this option creates an .xls file containing a sheet for each sample and channel (named *SampleName-ChannelName*). Each sheet exports the following data for dots (one dot per row)
 - Field Of View
 - Event ID
 - Dot ID
 - Dot Area
 - Dot Min Intensity
 - Dot Mean Intensity
 - Dot Max Intensity
 - Dot Sum Intensity

15. Segmentation Methods

TissueQuest application offers unique and proprietary algorithms for recognition of individual cells in a tissue context or a cell culture monolayer.

An **algorithm** is a sequence of mathematically realized processing steps applied to an image or part of an image with the aim of improving image quality and/or segmenting relevant objects (in this case: cells) in an image:

- discriminates background from specific cell objects;
- recognizes individual cells (where does one cell end and the neighboring cell begin);
- measures the marker intensity of each cell, as well as other parameters related to intensity, texture and morphology.

The starting point for cell identification is the segmentation of nuclei (e.g. object recognition and background rejection).

The segmentation algorithms require different parameters for every type of channel. The detection parameters for the channels are actually adjusted for the entire group of channels to which the channels belongs.

In the figure below, a comparison is shown of the default parameters for a master channel (left side) and a non-master channel (right side).

Figure 133 - Default parameters for a master channel (left)

and a non-master channel (right)

To **change the default value of a parameter**, simply modify it in the corresponding field.

15.1. Changing Segmentation Method

15.2. Various Shapes 1.0

TissueQuest provides a method especially created to detect structures with various shapes and sizes: **Various Shapes 1.0**. For tuning this method, a parameter set is available.

15.2.1 Master Channel Parameters



Nuclei Size:	5
Discrimination by Area:	2
Discrimination by Gray:	1
<input checked="" type="checkbox"/> Autodetect Background Threshold	
Threshold Level:	25

Figure 134 - Master channel parameters

Nuclei Size: specifies the size of the nuclei. This is an arbitrary/unitless value and it should be increased if your nuclei are oversegmented and decreased if the nuclei are undersegmented.

Discrimination by Area: this parameter removes the smallest objects from the measure mask (i.e. the result of the image segmentation). By increasing this parameter, more small events are removed from the result of the segmentation.

Discrimination by Gray: this parameter removes the objects with lowest staining intensity. By increasing this parameter, more events with low staining intensity are removed from the measure mask (i.e. the result of the image segmentation).

Autodetect Background Threshold: specifies if automatic detection of the background level should be used by marking the checkbox or can be set manually by unselecting the checkbox and providing the desired value in the **Threshold Level** field.

15.2.2 Non-Master Channel Parameters

☒ Ring Mask
 Interior Radius:
 Exterior Radius:

☒ Identified Cell Mask
 Max Growing:
 Offset from Nuclei:

☒ Autodetect Background Threshold
 Minimum Level:
 Maximum Level:
 Threshold Level:

☒ Include Nuclei Label

Figure 135 - Non-Master Channel parameters

Ring Mask: if you check this parameter, a ring mask around the objects detected in the master channel is created and included in the segmentation result.

Interior Radius and Exterior Radius: the **Interior radius** and **Exterior radius** parameters allow the user to define in which direction (outwards, inwards or both) and to what extent this ring should be created. The value represents the number of concentric pixel layers. As this ring construction takes care of neighboring objects and statistical growth process, it stops when the growing environment of a neighboring cell is reached (e.g. when cells touch each other). The values for radius represent the maximum growth. The interior radius ring growth process stops after the specified distance is reached or if the entire nucleus is covered.

Identified Cell Mask: by selecting **Identified cell mask** as measurement strategy, the nuclei are used as seeds (for example, starting points of image analysis and cell recognition). This algorithm searches for specific staining patterns in the other channels of one field of view and causes binary mask objects to “grow” along those specific structures.

Max Growing: The user may limit this growth process on demand by setting this parameter.

Offset from Nuclei: in order to reach the marker staining, some additional growing steps might be required. In this case, the growing process will not start immediately at the border of the nucleus, but outside, at a distance specified by this parameter.

Autodetect Background Threshold: specifies if automatic detection of the background level should be used by marking the checkbox or can be set manually by unselecting the checkbox and providing the desired values in the **Minimum Level**, **Maximum Level** and **Threshold Level** fields.

Include Nuclei Label: By selecting **Nuclei mask**, the area of the binary objects in the nucleus mask is also used as measurement mask in all other channels.

15.3. DOT Finder

15.3.1 DOT Parameters

To detect specific, dot-like features present in a sample (e.g. *FISH - Fluorescent In Situ Hybridization*), **TissueQuest** application provides an algorithm for dot detection. This algorithm will detect dots present in the virtual channel mask specified by the user. The DOT Finder can be applied to nuclear and/or cytoplasmic masks (FISH is a typical example for a nuclear application: dots in the cytoplasm might come from certain markers like signaling molecules or markers associated with mitochondria or markers associated with endoplasmatic reticulum).

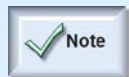
First, go to **Project** → **Virtual Channels...** and, in the dialog that appears, check a virtual channel (see **Chapter 6.4**). The name of the new virtual channel will look like this: [**Channel Name**] (**DOT**).

At this point, you will find the new virtual channel displayed at the end of the **Parameters** list.

The screenshot displays the 'Parameters' dialog box in TissueQuest, showing settings for three different channels. The 'DAPI' channel is expanded, showing parameters for Nuclei Size (24), Discrimination by Area (1), Discrimination by Gray (0), and an unchecked 'Autodetect Background Threshold' with a Threshold Level of 30. The 'Rhodamine' channel is also expanded, showing 'Ring Mask' (checked) with Interior Radius (1) and Exterior Radius (3), 'Identified Cell Mask' (checked) with Max Growing (30) and Offset from Nuclei (0), and 'Autodetect Background Threshold' (checked) with Minimum Level (0), Maximum Level (255), and Threshold Level (25). The 'Include Nuclei Label' checkbox is unchecked. The third channel, '49 DAPI (Dot)', is highlighted with a red box and shows a dropdown menu with options: Default, Small Dots, Large Dots, Small Conic Dots (selected), Large Conic Dots, Small Cylindric Dots, and Small Cylindric Dots.

Figure 136 - DOT Parameters

From the dropdown list (available by pressing the  button), select the template which best fits your needs.



- If no suitable template is available in the dropdown list, please contact TissueGnostics support.

After selecting the desired template, run the algorithm in order to obtain the results.

A series of output measurements will be available; they are described in **Chapter 17.2.2**

16. Image Analysis

When working with **TissueQuest** software, you have different possibilities to run detection, depending on your analysis needs.

1. Go to the menu bar of the application and choose **Algorithm**. A menu like in the image below will appear:

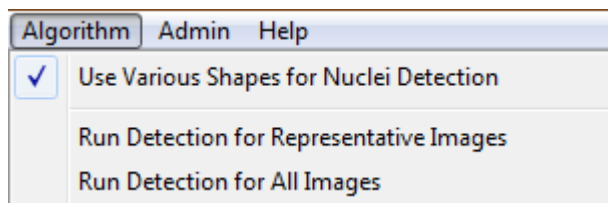




Figure 137 - Algorithm menu

In this menu, you can choose one of the two detection options:

- **Run Detection for Representative FOVs of a sample**
- **Run Detection for All Images**



- The same two options are available by going to the main toolbar and choosing one of the two buttons:

1.  **Run detection for representative images only**
2.  **Run detection for all images**

2. To **run the algorithm** for a specific **sample** or **field of view**, follow the next steps:

- Right click on the sample name or on the field of view name.
- A context menu will appear.
- Choose the desired option from the context menu:
 - **Run detection for Representative FOVs for this sample only** (right click on the sample name)
 - **Run detection for ALL FOVs for this sample only** (right click on the sample name)
 - **Run detection only for this Field of View** (right click on the FOV name)

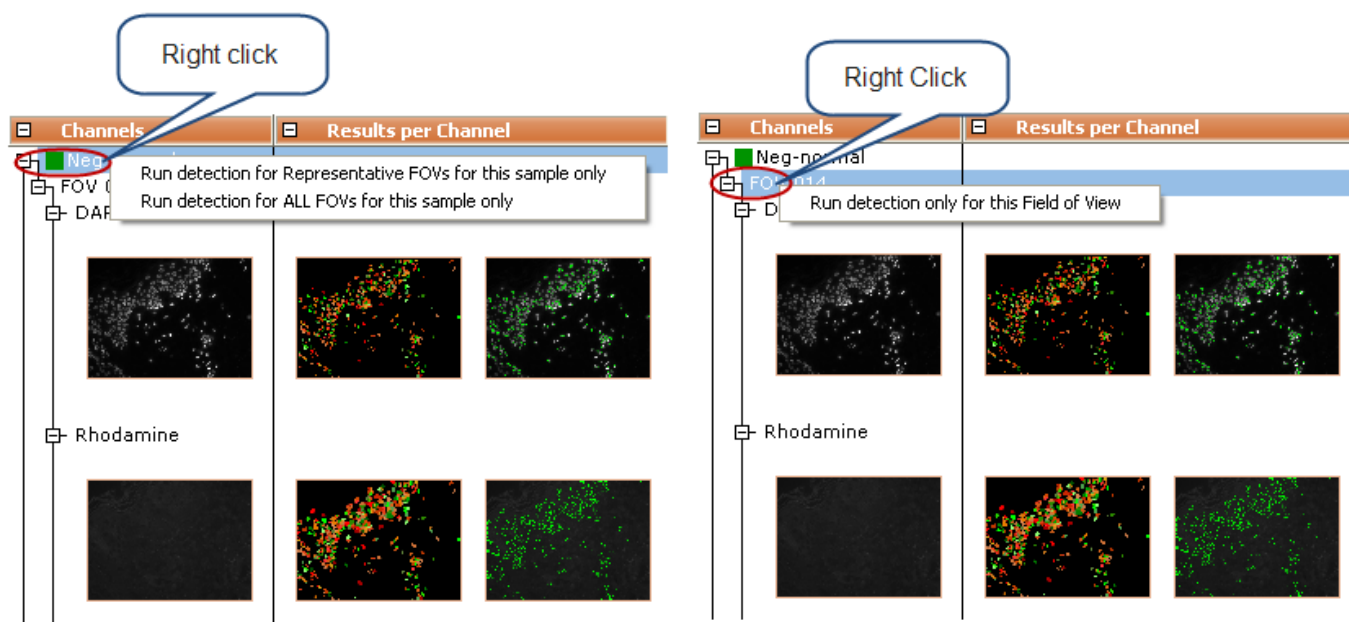


Figure 138 - Channels list: running the algorithm

While the algorithm is running, the following splash screen will be displayed, informing you about the progression of the process (elapsed time, number of processed FOVs):

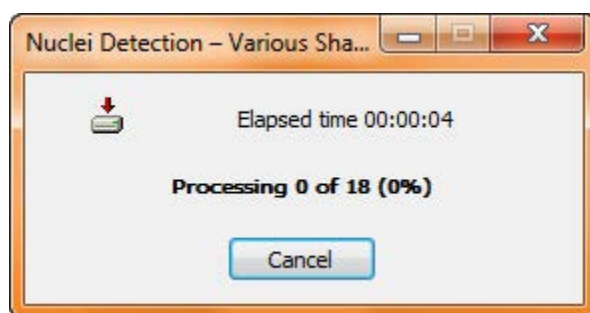


Figure 139 - Run Detection splash screen

17. Analysis Results

17.1. Image Results

After analyzing the samples and/or fields of view, the user will obtain for each channel a set of **image results**:

- **Labeled:** a pseudo-coloring of the events will be displayed. The colors are chosen randomly and do not indicate different staining intensities. They simply help showing the segmentation results.

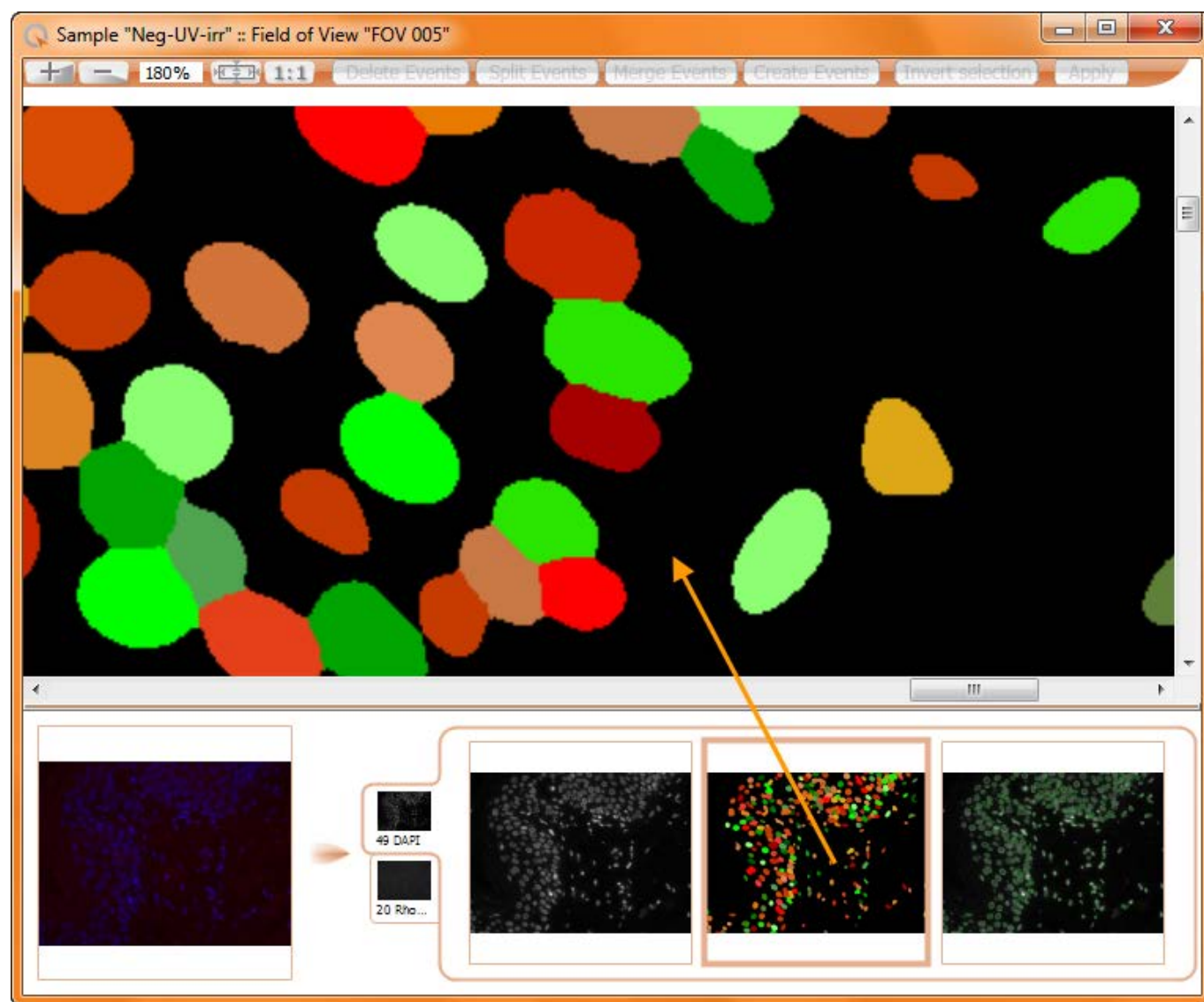


Figure 140 - Image Results: Labeled

- **Overlay:** the contours of the events will be visible on the displayed image.

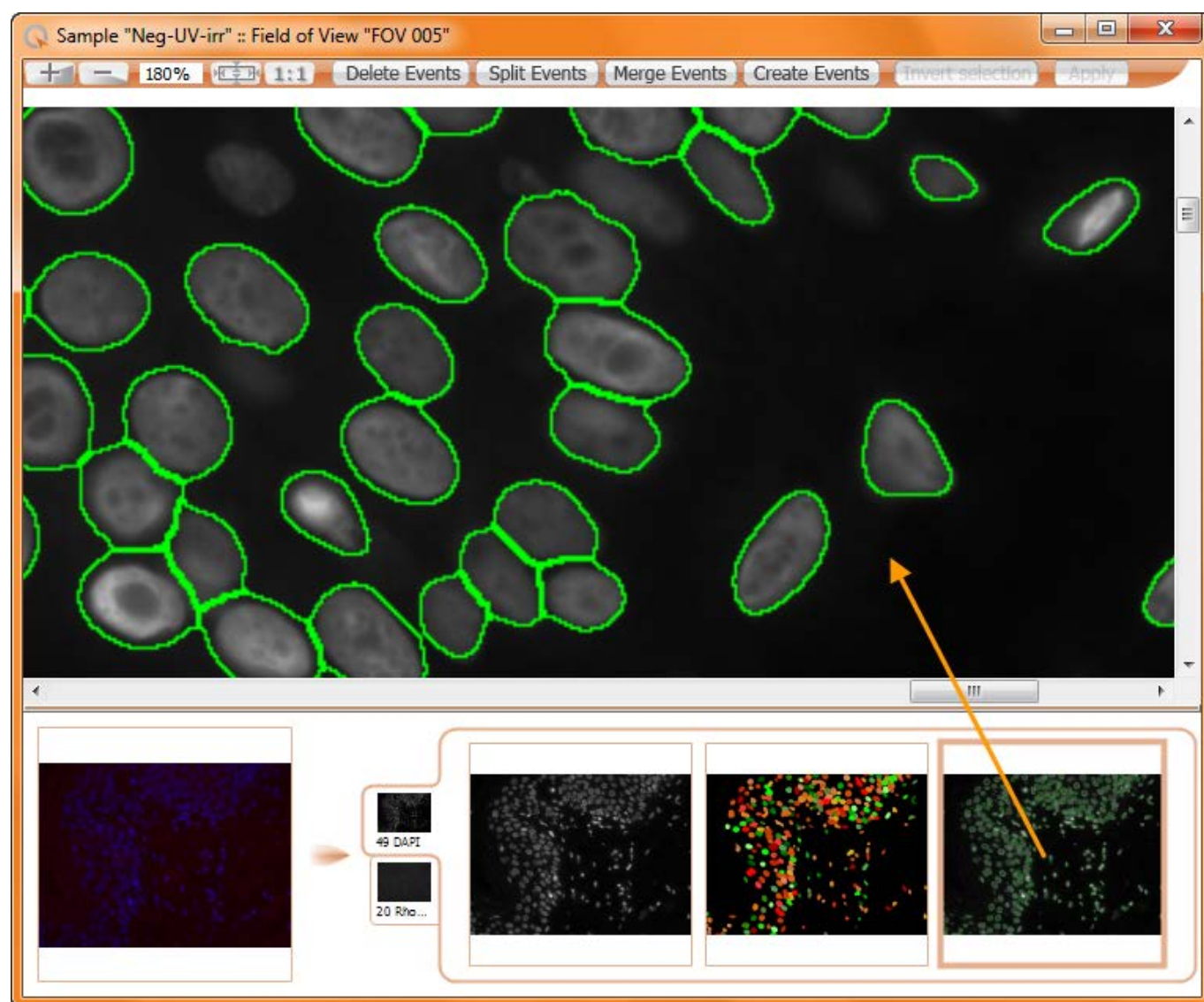


Figure 141 - Image Results: Overlay (right)


17.2. Raw Data

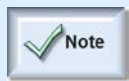
17.2.1 Raw Data for Various Shapes 1.0

For **Various Shapes 1.0**, the following output parameters are available for each detected event:

- **Area**: the area of the segmented object.
- **Minimum of Intensity**: minimum gray level expressed by the pixels of the object.
- **Maximum of Intensity**: maximum gray level expressed by the pixels of the object.
- **Range of Intensity**: the gray level interval (max-min).
- **Sum Intensity**: sum of all gray level intensities expressed by the pixels of the object.
- **Mean Intensity**: the average gray level expressed by the pixels of the object.
- **Equivalent Diameter**: the diameter of the disk which has the same area as the object.
- **Variance of Intensity**: the measure of the amount of variation of all the gray level intensities expressed by the pixels of the object.
- **STD of Intensity**: the average difference of the gray values from the mean of intensity.

- **Perimeter**: the perimeter of the segmented object.
- **Compactness**: it measures how much the segmented object shape resembles a disk. The more likely it resembles a disk, the expressed compactness is closer to 1. If not, its value will be closer to 0.
- **Eccentricity**: it measures how much the object shape resembles a circle or a line segment. When it is closer to 0, the object resembles a circle; when it is closer to 1, the object is more elongated.
- **Minimum Width**: minimum value a micrometer would express, taken into account all possible measurements positions (orientations).
- **Maximum Length**: maximum value a micrometer would express, taken into account all possible measurements positions (orientations).
- **Feret Ratio**: the ratio between minimum width and maximum width.

These output parameters are used in order to associate data to specific axis of the scattergram. To do this, access the **Scattergram properties** dialog from **Scattergram toolbar** (, see [Chapter 10.3](#)).



- **Scattergram properties** dialog is also available when adding a new scattergram from **Scattergram management** (see [Chapter 10.1.1](#)).

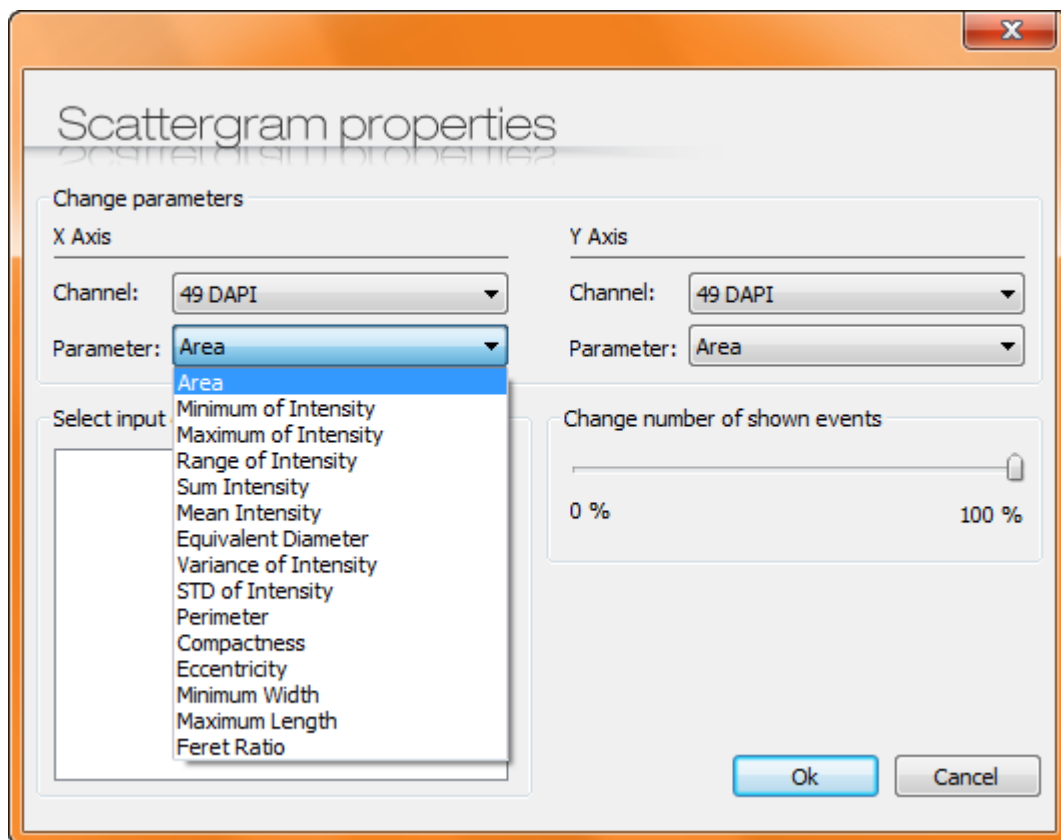




Figure 142 - Scattergrams: Scattergram properties dialog - Parameter list

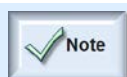
Press the arrow button () from the **Parameter** section to see the dropdown list containing the [raw data for Various Shapes 1.0](#) (parameters available after running the algorithm).

17.2.2 Raw Data for DOT Finder

For DOT detector, the following output parameters are available for each detected event:

- **Dot Count:** the number of dots present in a specified event.
- **Dot Mean Intensity:** the mean intensity (gray value) of the dots present in a specified event.
- **Dot Sum Intensity:** the sum of the intensities of the dots present in a specified event.
- **Dot Total Area:** the total area of the dots present in a specified event

These output parameters are used in order to associate data to specific axis of the scattergram. To do this, access the **Scattergram properties** dialog from **Scattergram toolbar** (, see [Chapter 10.3](#)).



- **Scattergram properties** dialog is also available when adding a new scattergram from **Scattergram management** (see [Chapter 10.1.1](#)).

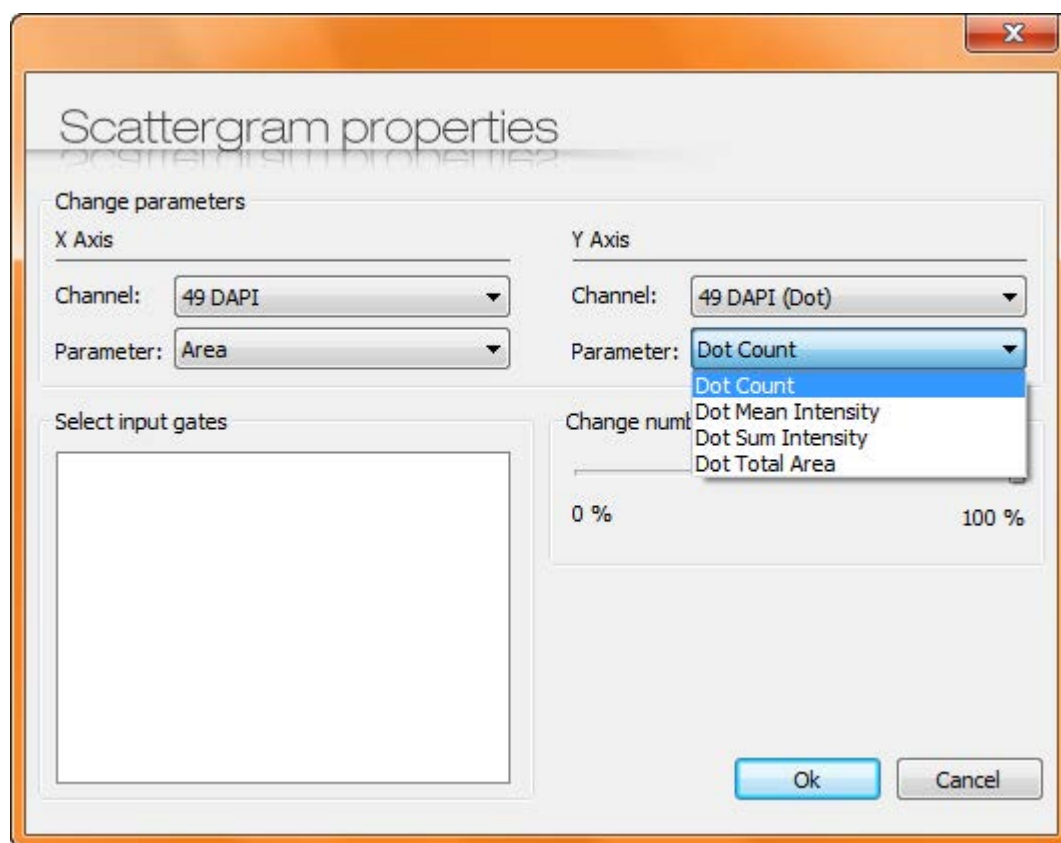


Figure 143 - Scattergrams: Scattergram properties dialog - DOT Parameter list

18. Admin menu

You can find the **Admin** menu in the Menu bar of the application.

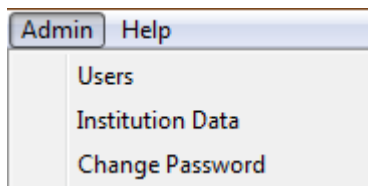


Figure 144 - Admin menu

18.1. Users

User management option is available only for the Administrator. The standard user is allowed only to change its password.

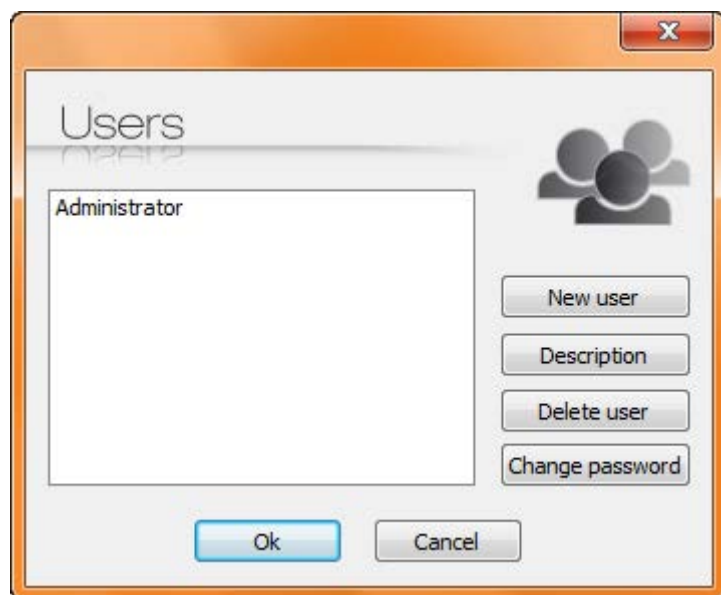


Figure 145 - Users dialog

In order to create a new user for **TissueQuest**, press **New user**, then enter the username into the **Username** field, respectively the password into the **Password** field. Then press **OK** to create the new user.

A dialog box titled "New User" with a close button (X) in the top right corner. The text inside says "Please type in the name for the new user and a password." There is a user icon in the top right. Below the text are three input fields: "Username:" with a single character "I" entered, "Password:", and "Repeat password:". At the bottom are "Ok" and "Cancel" buttons.

New User

Please type in the name for the new user and a password.

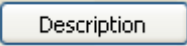
Username: I

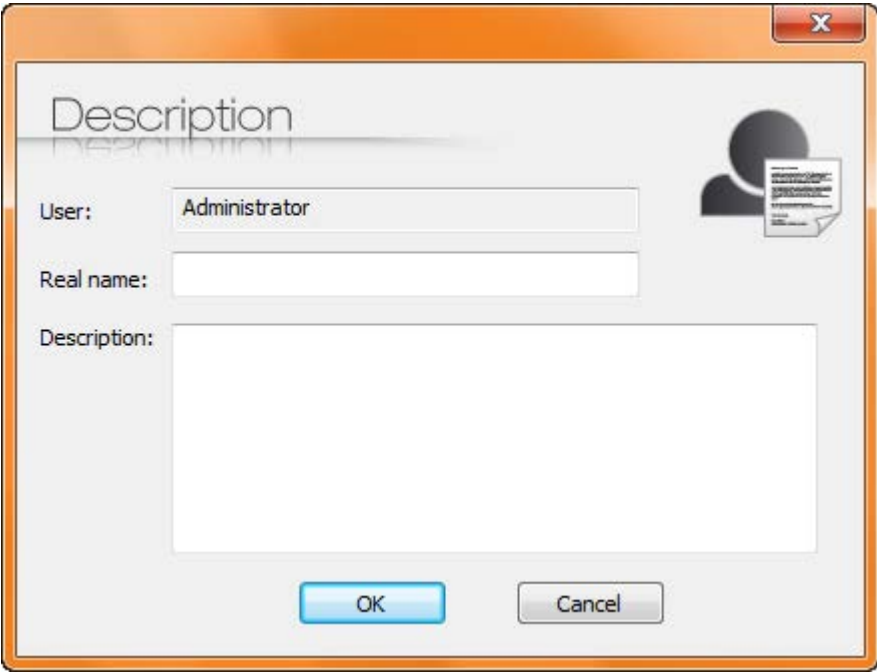
Password:

Repeat password:

Ok Cancel

Figure 146 - New User dialog

You can also add a description for each user, in the **Description** dialog box. Open the **Description** dialog box by pressing the **Description** button ().

A dialog box titled "Description" with a close button (X) in the top right corner. It features a user icon in the top right. The "User:" field is populated with "Administrator". Below it are "Real name:" and "Description:" (a large text area) fields. At the bottom are "OK" and "Cancel" buttons.

Description

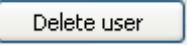
User: Administrator

Real name:

Description:

OK Cancel

Figure 147 - User Description dialog

To delete a user, select it, then press **Delete user** button (). A warning message like the one below will appear, asking you if you really want to delete that user.

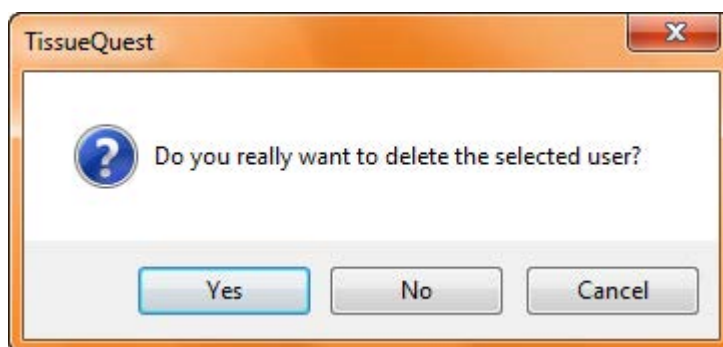


Figure 148 - Delete User dialog

To change the password of a user, select it, then press **Change password** button (Change password). The **Change password** dialog will appear.



Figure 149 - Change Password dialog (Administrator)

If you change the password of the **Administrator**, you must enter its current password.

If you change the password of a standard user, its current password is not required.

A standard Windows-style dialog box with an orange title bar and a close button (X) in the top right corner. The title is "New Password". Below the title is a user icon. The text "Define a new password." is displayed. There are three text input fields: "Current password:", "New password:", and "Repeat password:". At the bottom are "OK" and "Cancel" buttons.

New Password

Define a new password.

Current password:

New password:

Repeat password:

OK Cancel

Figure 150 - Change Password dialog (standard user)

18.2. Institution Data

A standard Windows-style dialog box with an orange title bar and a close button (X) in the top right corner. The title is "Institution Data". Below the title is a building icon. The text "Enter the institution's data." is displayed. There are three text input fields: "Institution name:", "Department:", and "Laboratory:". At the bottom are "Ok" and "Close" buttons.

Institution Data

Enter the institution's data.

Institution name:

Department:

Laboratory:

Ok Close

Figure 151 - Institution Data dialog

In this dialog, you can provide data concerning your institution:

- Institution name
- Department
- Laboratory

18.3. Change Password

To change the password, you have two options:

- Go to application main Menu, then choose **Admin** → **Change Password**.

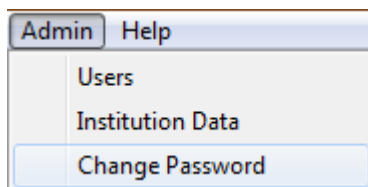


Figure 152 - Change Password: Admin Menu

The following dialog will appear:



Figure 153 - New Password dialog

To change the password, you must enter the current password into the **Current password** field, then enter the new password into the **New password** field and also, for confirmation, into the **Repeat password** field.

19. Help menu

By going to **Help** → **About TissueQuest...**, you will see displayed a splash screen containing the main information about **TissueQuest** version in use.

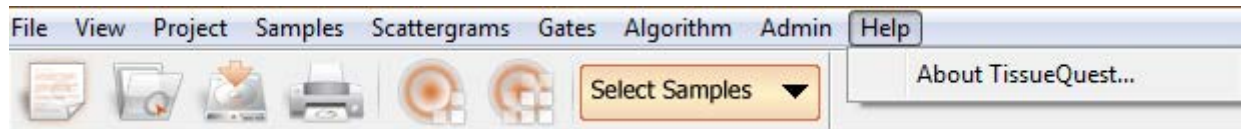


Figure 154 - Help menu

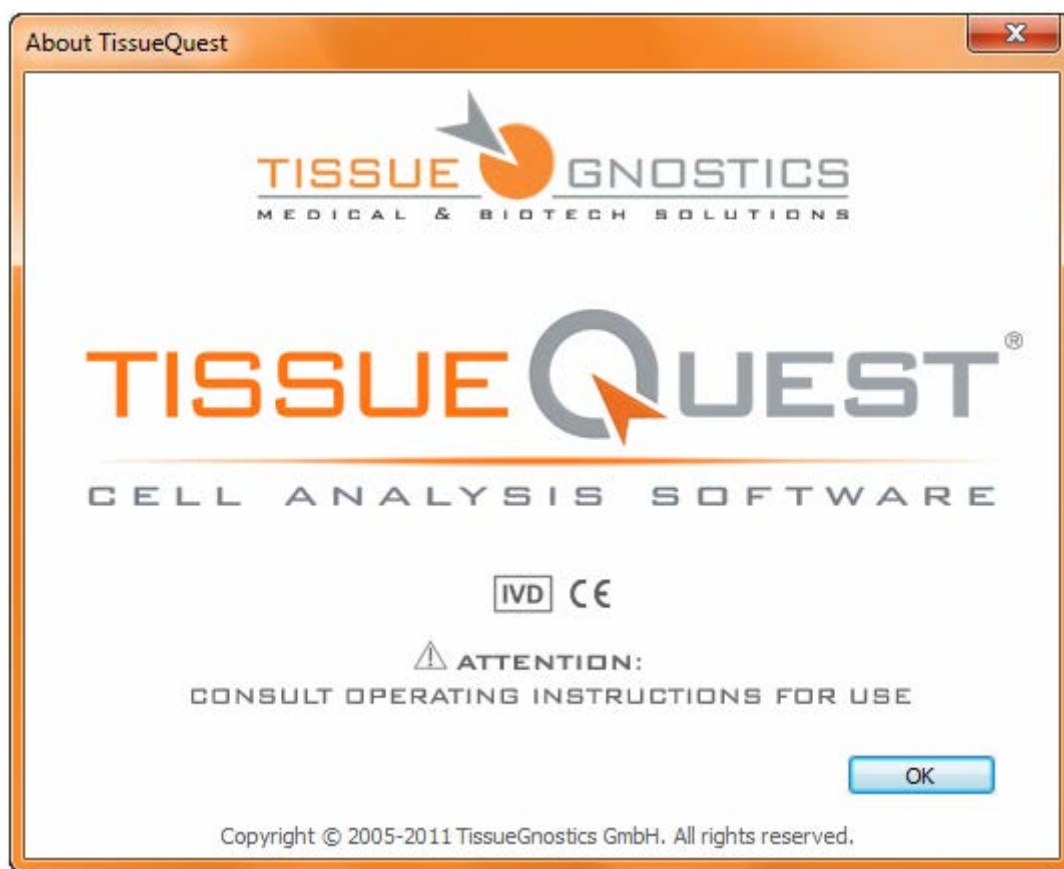


Figure 155 - Help menu: Splash screen

20.